THE UNIVERSITY OF ALBERTA

PHOTOENDOCRINE RESPONSES AND PHOTORECEPTION IN JAPANESE QUAIL (*Coturnix coturnix japonica*)

by

© TADASHI OISHI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

FALL, 1972

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled PHOTOENDOCRINE RESPONSES AND PHOTORECEPTION IN JAPANESE QUAIL (*Coturnix coturnix japonica*) submitted by TADASHI OISHI in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

I. Mature male Japanese quail (*Coturnix coturnix japonica*) reared under continuous light or long photoperiods have gonads of maximal size, which then regress when the birds are transferred to a short photoperiod or to darkness. This photoperiodic testicular response has been studied with respect to the location of the photoreceptor(s), the effect of changes in light intensity, and the action spectrum for gonadal maintenance.

The photoreceptor(s) for the photoperiodic testicular response is shown to be restricted to the head region. Since the response of the testes to light was observed after removal of the eyes, the pineal body and the Harderian glands, the brain itself (probably the hypothalamus) seems to be the major photoreceptor. The possible function of the eyes and the pineal body as auxiliary photoreceptors or as light guides is suggested. The Harderian gland does not seem to be a photoreceptor in Japanese quail.

The light intensity threshold of white light necessary to maintain maximal gonadal size in both intact and enucleated adult male quail was determined to be approximately 1.57 - 1.67 $\mu w/cm^2$. In intact birds, the effective portions of the spectrum for maintenance of maximal gonadal size were red (625 nm) and green (500 nm)

but not blue (450 nm) when the light was dim (4.0 - 9.6 $\mu w/cm^2$), while red light was effective but green and blue light were ineffective at one tenth of this intensity (x 0.1 dim light). In enucleated birds, the action spectrum under dim light was the same as for intact birds at x 0.1 dim light.

Six factors (energy of light, number of photons, wavelength, photoperiod, absorption of light by tissues, site of photoreceptor) are discussed in an effort to interpret these results.

II. Endocrine organ weights (pituitary, adrenal and gonad) were higher in birds maintained under continuous light (24L/0D) than in a short photoperiod (8L/16D) in 5-week old quail after 3 weeks of lighting treatment. These responses of pituitary and adrenal weight to the photoperiod were abolished by pinealectomy in both males and females, while gonadal weight was not affected by pinealectomy. There was no effect of either the photoperiod or pinealectomy on body weight and thyroid weight.

Pineal hydroxyindole-O-methyltransferase (HIOMT) activity of adult birds was high in light and low in darkness.

Hypophysectomy caused marked atrophy of the gonads and adrenals of young birds and atrophy of gonads but not

adrenals in older birds, while body weight, pineal weight, pineal HIOMT activity and thyroid weight were not altered by hypophysectomy.

Three types of cells were identified electron microscopically in the parenchyma of the pineal: photoreceptor cells (pinealocytes), supportive cells (glial cells), and nerve cells (ganglion cells). Both rudimentary photoreceptor structures and secretory granules (800 - 1,200 Å in diameter) were observed in the photoreceptor cells. There was a greater number of secretory granules in the pericapillary area under continuous light than under continuous darkness. Half depleted and completely depleted dense-cored membrane-limited vesicles were frequently observed in pineal nerve endings under continuous darkness.

ACKNOWLEDGEMENT

I am greatly indebted to Dr. J. K. Lauber for her guidance and help throughout the execution of this study, and to Dr. M. Kato, Dr. A. Steiner, Dr. J. E. Boyd, and Mr. M. Uddin for the encouragement they gave me. It is a pleasure to express my thanks to Mr. J. Vriend and members of my supervisory committee for their valuable criticisms and suggestions, and to the staff of the electron microscope laboratory of the Faculty of Science for their kindness. I am thankful to the staff of Biosciences Animal Services for their help in animal care.

Financial support was afforded by a University of Alberta Graduate Research Assistantship and a Graduate Teaching Assistantship in the Department of Zoology of the University of Alberta. This project was funded in part by a research grant to Dr. J. K. Lauber from the National Research Council of Canada (#A-3446).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

I. GENERAL INTRODUCTION

Homeostatic control of the "milieu intérieur" resides in both the nervous and endocrine systems. The internal environment is not static, however, but is responsive to changes in the external environment. Light is one of the most important and well investigated aspects of the external environment affecting animals.

The eye has been considered to be the primary photoreceptor, and this is true in the case of vision. However, an increasing number of studies suggest that, even in higher vertebrates, the eye is not necessary for some responses to light, such as the photoperiodic gonadal response in birds (Benoit, 1964; Menaker, 1971). Thus, the location and nature of possible photoreceptors for the photoperiodic testicular response of Japanese quail have been studied.

The mammalian pineal body, which was once considered to be a rudimentary organ, has attracted renewed interest recently because now it is considered to be a neuroendocrine transducer for light information and possibly for some other environmental variables (Wurtman and Anton-Tay, 1969; Quay, 1970). Collin (1971) pointed out that the pineal photoreceptor cells have both photoreceptor and secretory functions in lower vertebrates. The

phylogenetic trend is toward loss of the photoreceptor function and increased secretory function in higher vertebrates. The avian pineal is of particular interest because birds occupy an intermediate position between mammals and lower vertebrates. Thus, the pineal of Japanese quail has been studied in its relation to the photoenvironment, and to several endocrine glands.

The Japanese quail (*Coturnix coturnix japonica*) was the experimental animal of choice because of its extreme sensitivity to the photoperiod, its short life span (reaching maturity in 6 weeks), its resistence to disease, and the ease of handling of this laboratory animal.

II. GENERAL MATERIALS AND METHODS

Japanese quail (Coturnix coturnix japonica), hatched from stock maintained at this laboratory for several years, were reared in floor pens under continuous incandescent illumination until the beginning of each experiment. Brooder heat was supplied during the early weeks. During experiments, which began at the ages indicated, adult birds were housed in environment chambers measuring 24" x 24" x 16" high, and incorporating suitable light, temperature and ventilation controls. Food (commercial turkey starter) and water were supplied ad libitum.

The photoperiod was controlled by timeclocks*. Incandescent illumination was provided by a 7.5 watt bulb mounted in the top of each environment chamber. The light energy at bird height was 156 $\mu w/cm^2$ (= 26 ft. c. = 260 lux). Reduced light intensities were achieved by neutral density filters. The light source and coloured filters used for action spectrum studies are described in Section III-B. Light energy was measured with an ISCO model SR spectroradiometer**. Body weights were measured on a

---

* Paragon Electron Co., Two Rivers, Wisconsin

** Instrumentation Specialties Co., Lincoln, Nebraska

triple beam Dial-O-Gram scale***, and for organ weights a precision torsion balance****, sensitive to 10 mg, and a precision balance*****, sensitive to 0.01 mg were used. Animal surgery was performed under Nembutal (Sodium pentobarbital) anesthesia (0.15 ml/100 gm body weight). All experiments in Section III were based on the observation that adult birds reared under a long photoperiod or continuous light have fully developed testes, and the gonads regress when a short photoperiod is applied. For statistical analysis of the data, Student's t test was used.

*** OHAUS Scale Co., Union, New Jersey

**** The Torsion Balance Co., Montreal, Quebec

***** Federal Pacific Electric Co., Newark, New Jersey

III. PHOTORECEPTION IN THE PHOTOPERIODIC TESTICULAR RESPONSE OF JAPANESE QUAIL

A. THE LOCATION OF THE PHOTORECEPTOR(S)

INTRODUCTION

The problems which are involved in the study of photoreception are, 1) the site of the photoreceptor(s), 2) the action spectrum of the response, 3) the effective intensity of light to elicit the response. In this section, I have concentrated on the location of the photoreceptor for the response of the testes to changing environmental photoperiod, a phenomenon which is called the photoperiodic gonadal response. A specific phase of this response was chosen for this study, that is, the maintenance of mature testes under a long photoperiod or continuous light, and the regression of the testes under a short photoperiod or continuous darkness.

The eye is a highly specialized photoreceptor for vision. However, it is apparently not the sole photoreceptor of the body; many reports indicate the existence of extraocular photoreceptors in various phyla of animals. Electrophysiological investigations in invertebrates revealed that the abdominal ganglion in crayfish (Kennedy, 1963), the pallial nerves of a lamellibranch (Kennedy, 1961), and the radial nerves in an echinoid (Yoshida and

Millott, 1959) respond to light. Histological investigation in squids demonstrated extraocular photoreceptors (Baumann _et al_, 1970). In insects, the brain seems to be the photoreceptor for photoperiodically controlled diapause (De Wilde _et al_, 1959; Williams _et al_, 1965), and for the production of sexual and parthenogenetic females in aphids (Lees, 1964).

In lower vertebrates, electrophysiological studies have shown that the pineal body and/or parapineal body, which has a retina-like structure, responds directly to illumination (Dodt, 1963; Morita, 1966 a; Hanyu _et al_, 1969, in fish; Dodt and Heerd, 1962; Dodt and Jacobson, 1963; Morita and Dodt, 1965; Dodt and Morita, 1967, in amphibians; Miller and Wolbarsht, 1962; Dodt and Scherer, 1968; Hamasaki, 1968, in reptiles). As cited below, physiological observations also have shown that there is an extraretinal photoreceptor(s) in lower vertebrates. The pineal body seems to be a photoreceptor for light-induced chromatophore contraction in cyclostomes (Young, 1935; Eddy and Strahan, 1968). Extraretinal photoreception is reported to be involved in vertebrate phototaxis and/or pigmentary responses (Fenwick, 1970; Hafeez and Quay, 1970, in fish; Mrosovsky and Tress, 1966; Taylor and Ferguson, 1970, in amphibians), and in circadian locomotor rhythms entrained

by the photoperiod (Adler, 1969, 1970, in amphibians; Underwood and Menaker, 1970 b in reptiles). Clausen and Mofshin (1939) identified pineal and dermal photoreceptors in lizards on the basis of differences in the rate of oxygen consumption under dark and light conditions.

Extraretinal photoreceptors have been suggested even in mammals (Lisk and Kannwischer, 1964, in adult rats; Zweig _et al_, 1966; Snyder, 1968, in neonatal rats), although the eyes seem to be the primary photoreceptor (Moore, 1969). Wetterberg _et al_ (1970 a, b) reported that the Harderian gland is an extraretinal photoreceptor which participates in photoperiodically controlled serotonin and HIOMT rhythms in the pineal body of neonatal rats.

In birds, Benoit (1935 b, c) was one of the first to suggest the existence of extraretinal photoreceptors for the photoperiodic gonadal response. Benoit and his colleagues concluded that both the eyes and a deep photoreceptor (hypothalamus ?) participate in the photoperiodic gonadal response in ducks (Benoit, 1964). The existence of an extraretinal photoreceptor(s) has been confirmed for several species of birds since Benoit's pioneer work (Ishibashi, 1957; Lauber _et al_, 1968; Ookawa, 1970 a, b, in the chicken; Oishi _et al_, 1966; Sayler and Wolfson, 1968 b, in Japanese quail; Menaker and Keatts, 1968; Under-

wood and Menaker, 1970 a, in the house sparrow; Rosner et al, 1971, in the duck). Consequently, extraretinal photoreception seems to have an important role in the photoperiodic responses of animals, as Menaker and his colleagues emphasized in several papers (Menaker, 1968, 1971; Menaker and Keatts, 1968; Menaker et al, 1970; Underwood and Menaker, 1970 a). The photoreceptor(s) for photoperiodic gonadal responses of birds seems to be restricted to the head region: gonadal activity was suppressed when the whole head was covered with a black cloth (Benoit, 1935 a, 1937, in the duck; Oishi, 1967, in Japanese quail; Ringoen and Kirschbaum, 1938, in the English sparrow), although an exception was reported by Ivanova in the house sparrow (1935). Several possible photoreceptors have been suggested: the eye (Benoit, 1964), the hypothalamus (Benoit, 1964; Lisk and Kannwischer, 1964), the pineal body (Oishi and Kato, 1968; Munns, 1970; Rosner et al, 1971), and the Harderian gland (Wetterberg et al, 1970 a, b).

MATERIALS AND METHODS

Experiment I: This experiment was designed to confirm the location of the photoreceptor(s) in the head region and to check the effect of time of feeding, which must be restricted when the head is covered by a hood.

The outline of the experiment is shown in Figure 1. Adult male quail (6 week old) reared under continuous light (24L/OD) were divided into two groups. For group A (control), food was supplied for only 8 hours, between 0800 and 1600; in group B, the head was covered with a hood, made from black cloth, for 16 hours, between 1600 and 0800, and food was supplied between 0800 and 1600. Both A and B groups were kept in continuous light for two weeks. All the birds were killed by decapitation, and body weights and testis weights were measured. Temperature in the chambers was 31.6 ± 0.2°C. Light intensity was 157 $\mu w/cm^2$.

Experiment II: This experiment was designed to test the existence of an extraretinal, extrapineal photoreceptor. The outline of the experiment is shown in Figure 3. Adult male quail (6 or 8 week old) reared under 14L/10D were put in continuous light (24L/OD) for 2 weeks and then divided into 7 groups. Groups A and B represented unoperated controls. In C and D groups, the eyes were removed and sham pinealectomy was performed. In groups E and F, both the eyes and the pineal body were removed. In group G, the eyes and the pineal body were removed and the eye sockets were coated with black gum arabicum. Group A, C, E, and G birds were kept in continuous light

(24L/OD); group B, D, and F birds were kept in a short photoperiod (8L/16D) for 22 days, at which time the experiment was terminated for all groups.

Enucleation of one eye was done 13 days before the start of the experiment, and the other eye was removed 2 days later. After removal of the eyeball, cotton was used to control bleeding. Pinealectomy or sham operation was done 7 days before the start of the experiment. For pinealectomy, the skin on the top of the head was cut to expose the skull. An incision (0.25 cm^2) in the skull above the pineal body was cut with a dental drill and a scalpel. The pineal body was removed with forceps through a hole made in the meninges. Fibrin foam (Gelfoam*) was placed on the wound to stop bleeding, and the skin was drawn together with two wound clips.

Light intensity was 15.7 $\mu w/cm^2$ (2.8 ft. c., 26 lux), which was one tenth of the intensity used in Experiment I. Temperature was 30.1 ± 0.6 °C.

Experiment III: This was a preliminary experiment designed to check whether or not the Harderian gland is a photoreceptor for the photoperiodic gonadal response. The details are shown in Figure 5. Adult male quail (15

---

* Upjohn Company of Canada, Don Mills, Ontario

week old) were divided into 4 groups. A and B groups served as controls. Eyes were removed from groups C and D birds. Harderian glands were removed as well from birds in group D. Groups A, C, and D were kept in continuous light and group B birds were kept in a short photoperiod (8L/16D). After 6 weeks of the experiment, birds in group A, B and C were killed, and body weights and testis weights were measured. In group D, the size of the cloacal gland was checked and then the birds were divided into two subgroups (E and F). Pineal bodies of the group F birds were removed. Both E and F groups were kept in continuous light for another 17 days until termination of the experiment. Temperature was 28.9 ± 0.6 °C. Light intensity was 157 $\mu w/cm^2$.

RESULTS

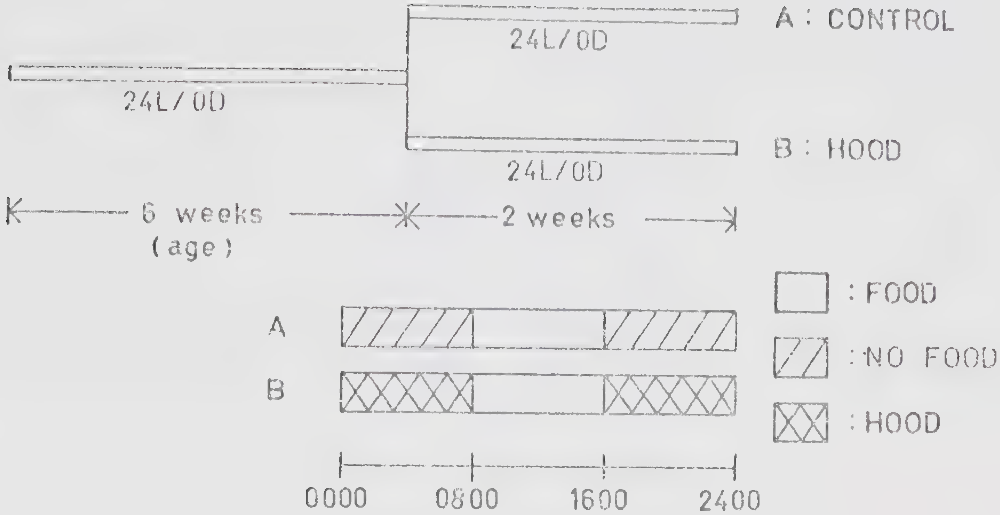
Experiment I: The results are shown in Table I and Figure 2. Group A birds (under 24L/OD, without hoods and with a restricted feeding period) maintained mature testis weight (1,203 ± 119 mg). On the other hand, group B birds (under 24L/OD, with hoods and with a restricted feeding period) showed considerably reduced testicular weight (222 ± 62 mg). The difference was highly significant ($p < 0.001$). Consequently, the assumption that a restricted feeding period might have an effect on gonadal activity

can be discarded. The results confirmed that the photoreceptor(s) for the photoperiodic gonadal response is in the head region.

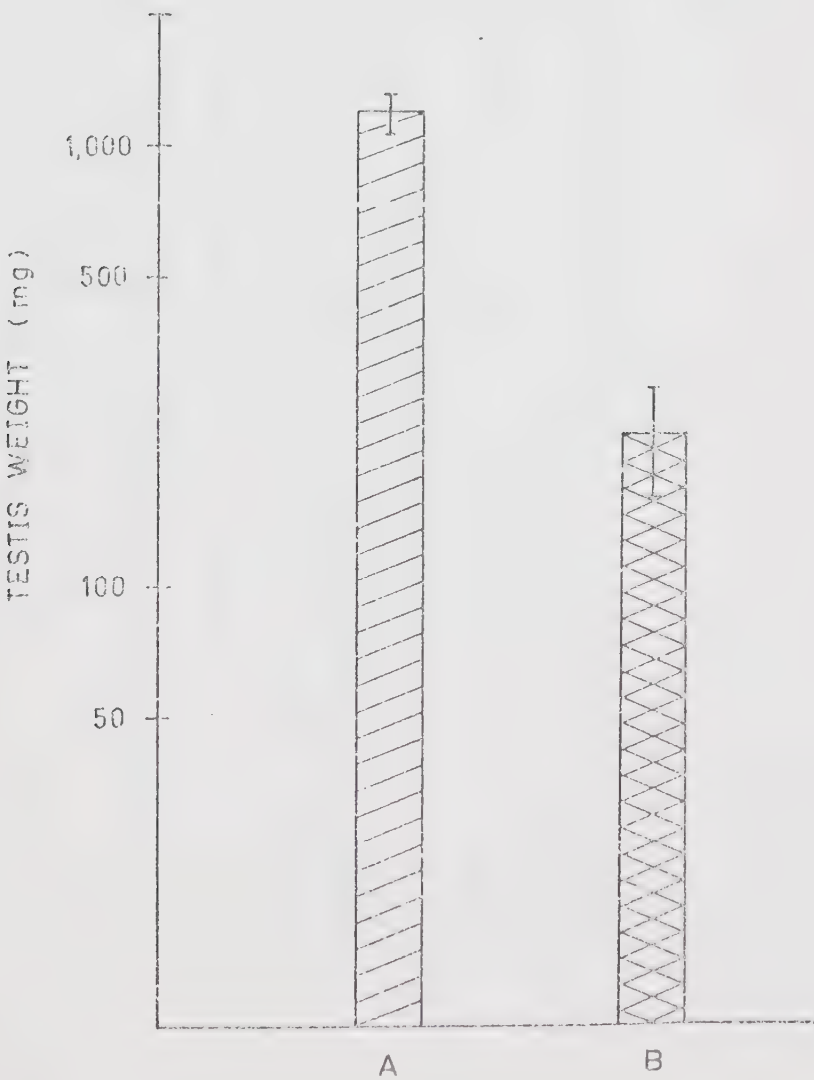
Experiment II: The results are shown in Table II and Figure 4. Group A birds (24L/0D, control) maintained testis weight (1,483 ± 66 mg) as expected. Birds in group B (8L/16D, control) showed drastically reduced testis weight (695 ± 205 mg; $p < 0.01$), after the 22 days of the experimental period. Although both eyes were removed, testis weights were maintained by group C birds (24L/0D), and decreased in group D birds (8L/16D). The difference between group C and D is not statistically significant, but this seems to be due to the small number of birds in group C (a number of these quail died after surgery). However, it is still appropriate to conclude that birds are able to receive light without the eyes, since group C birds maintained testis weights equal to those of groups A and E birds and testes of group D birds were significantly smaller than those of groups A and E birds ($p < 0.02$, and $p < 0.05$, respectively). After pinealectomy and enucleation, group E birds maintained high testis weight and group F birds showed reduced testis weight. The difference is statistically significant ($p < 0.05$). Group G birds (pinealectomized and eye sockets

Figure 1. Design of Experiment I (Section III-A) to confirm the existence of the photoreceptor(s) in the head region.

Figure 2. Existence of the photoreceptor(s) only in the head region. (Results of Experiment I in Section III-A).
The vertical bar represents standard error.



A : CONTROL
24L/0D
24L/0D
B : HOOD
24L/0D
6 weeks
(age)
2 weeks
A
B
: FOOD
: NO FOOD
: HOOD
0000
0800
1600
2400



TESTIS WEIGHT (mg)
1,000
500
100
50
A
B

Table I

Existence of the photoreceptor(s) only ir the head region.

(Results of Experiment I, Section III-A)

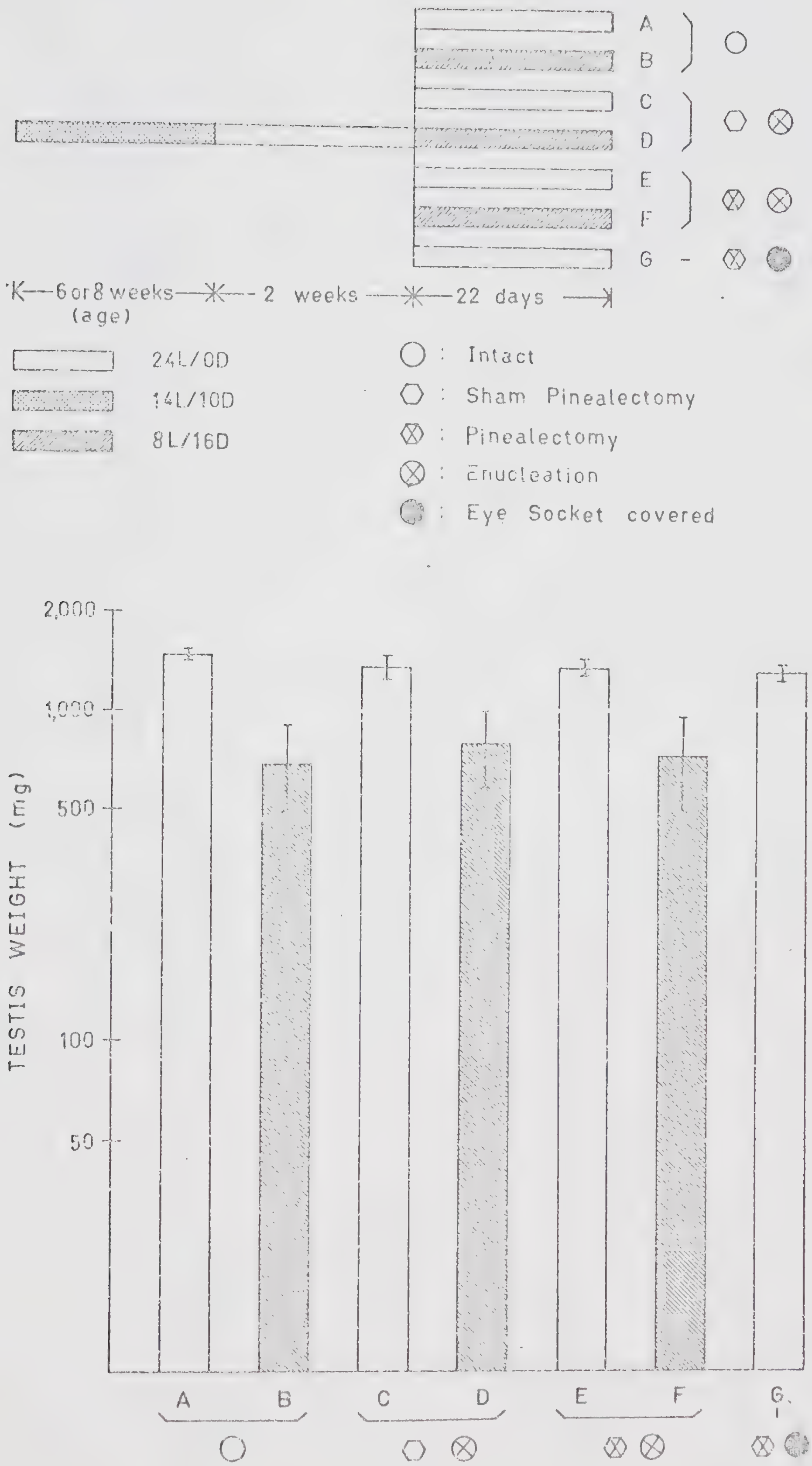
Treatment	Photoperiod	Number of Birds	Body Weight (gm ± S.E.)	Testis Weight (mg ± S.E.)
A (Cont.)	24L/OD	7	85.1 ± 2.3	1,203 ± 119 *
B (Hood)	24L/OD	7	92.6 ± 2.3	222 ± 62

* $p < 0.001$

S.E.: Standard error.

Figure 3. Design of Experiment II (Section III-A) to test the existence of an extraretinal, extrapineal photoreceptor.

Figure 4. Existence of an extraretinal, extrapineal photoreceptor (Results of Experiment II in Section III-A).
The vertical bar represents standard error.



A
B
C
D
E
F
G
6 or 8 weeks (age)
2 weeks
22 days
24L/0D
14L/10D
8L/16D
Intact
Sham Pinealectomy
Pinealectomy
Enucleation
Eye Socket covered
TESTIS WEIGHT (mg)
2,000
1,000
500
100
50
A
B
C
D
E
F
G

Table II

Existence of an extraretinal, extrapineal, photoreceptor.

(Results of Experiment II, Section III-A)

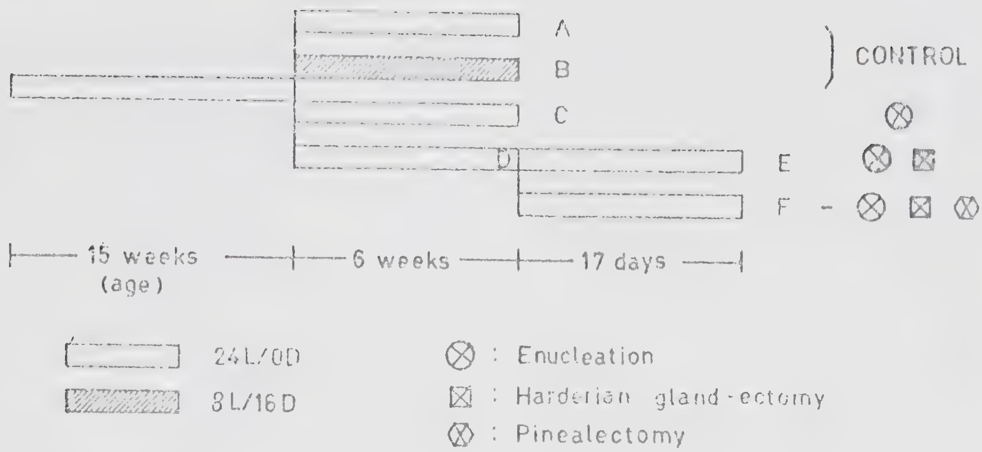
Group	Photoperiod	Number of Birds	Body Weight (gm ± S.E.)	Testis Weight (mg ± S.E.)
Intact Control				
A	24L/OD	10	114.2 ± 4.1	1,483 ± 66
B	8L/16D	11	108.9 ± 3.6	695 ± 205
Sham pinealectomy + Enucleation				
C	24L/OD	4	97.5 ± 10.5	1,364 ± 127
D	8L/16D	8	93.4 ± 5.5	800 ± 212
Pinealectomy + Enucleation				
E	24L/OD	8	97.3 ± 2.4	1,371 ± 79
F	8L/16D	8	97.5 ± 3.2	739 ± 232
Pinealectomy + Eye socket covered				
G	24L/OD	9	101.6 ± 4.0	1,321 ± 86

Testis Weight: A:B $p < 0.01$; C:D $0.05 < p < 0.1$; E:F $p < 0.05$; B:G $p < 0.05$; A:D $p < 0.02$; A:F $p < 0.02$; D:E $p < 0.05$

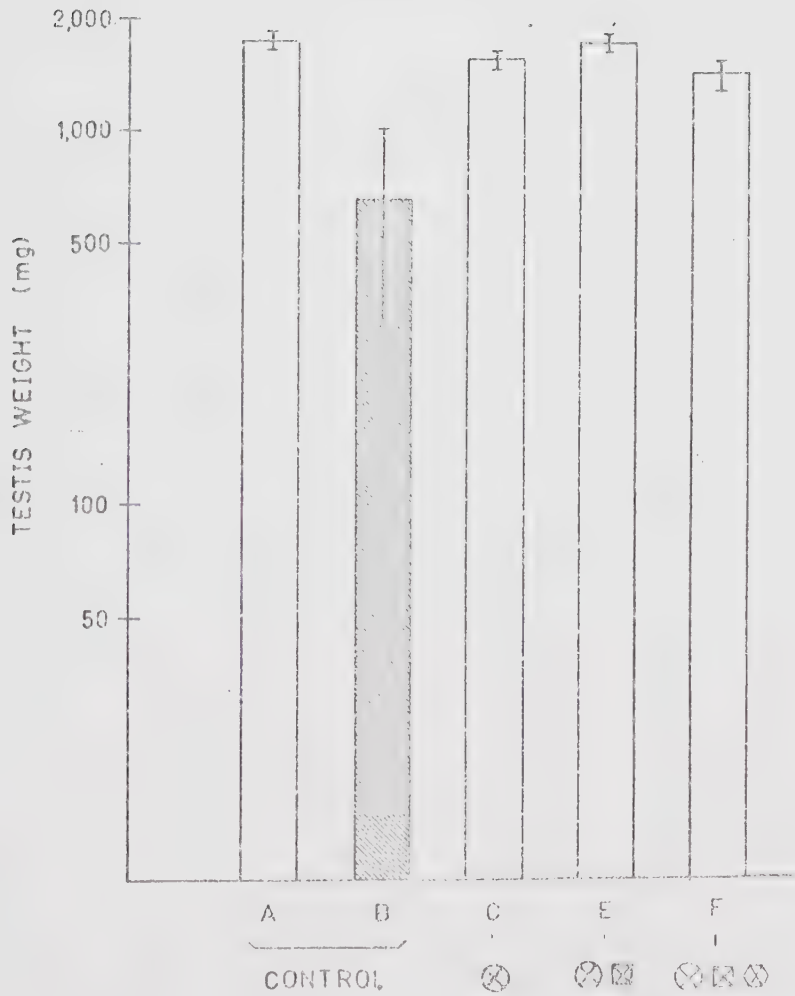
S.E.: Standard error.

Figure 5. Design of Experiment III (Section III-A) to test the existence of an extraretinal, extra-pineal, extra-Harderian gland photoreceptor..

Figure 6. Existence of an extraretinal, extrapineal, extra-Harderian gland photoreceptor. (Results of Experiment III in Section III-A).
The vertical bar represents standard error.



A
B
C
D
E
F
CONTROL
15 weeks
(age)
6 weeks
17 days
24L/0D
8L/16D
: Enucleation
: Harderian gland-ectomy
: Pinealectomy



TESTIS WEIGHT (mg)
2,000
1,000
500
100
50
A
B
C
E
F
CONTROL

Table III

Existence of an extraretinal, extrapineal, extra-Harderian gland photoreceptor.

(Results of Experiment III, Section III-A)

Treatment	Photoperiod	Number of Birds	Body Weight (gm ± S.E.)	Testis Weight (mg ± S.E.)
Control				
A	24L/OD	5	126.0 ± 5.8	1,742 ± 105
B	8L/16D	5	119.8 ± 3.4	661 ± 365 *
Enucleation of the eyes				
C	24L/OD	4	121.5 ± 3.9	1,578 ± 98
Enucleation of the eyes + Harderian gland ectomy				
D	24L/OD	10		big cloaca
E	24L/OD	5	119.0 ± 3.8	1,763 ± 118
Enucleation of the eyes + Harderian gland ectomy + Pinealectomy				
F	24L/OD	4	121.3 ± 5.7	1,446 ± 148

* $p < 0.05$

covered) maintained high testis weight as groups A, C and E birds did. These results indicate that light can be received without the eyes and the pineal body, and suggest that light may penetrate through the skull to stimulate some extraretinal, extrapineal photoreceptor. Body weights of groups D, E, and F quail were significantly lower than those of group A. This could be because the birds had difficulty in finding food after enucleation of the eyes. However, the above results are the same, whether the data are expressed as absolute gonadal weights or gonado-somatic index (T. W./B. W.).

Experiment III: The results are shown in Table III and Figure 6. The blinded birds in group C maintained high testis weights (1,578 ± 98 mg) after 6 weeks of the experimental period as group A (control) birds did. These results thus confirm the results of Experiment II. The cloaca in group D birds was enlarged and hyperemic, indicating full sexual activity. Subsequently, these birds were able to maintain their testis weights in the absence of both the eyes and Harderian glands. Group E birds had high testis weights (1,763 ± 118 mg) at 59 days after removal of the eyes and Harderian glands. Group F birds had also maintained their testis weights (1,446 ± 148 mg) 59 days after removal of the eyes and Harderian glands and

17 days after pinealectomy. These results indicate that there is a photoreceptor(s) for the photoperiodic gonadal response, other than the eyes, Harderian glands or the pineal body. It seems reasonable to assume that this receptor may be in the hypothalamus.

DISCUSSION

The results of Experiment I, and several other reports (Benoit, 1937, Ringoen and Kirschbaum, 1938) strongly suggest that the photoreceptor(s) for the photoperiodic gonadal response is restricted to the head region. There remains the possibility that, when the birds are hooded, the necessary restriction of feeding time, or the mechanical stimulus of being hooded, might somehow affect the gonadal response. However, the results of Experiment I, and others (Oishi, 1967) negate this assumption. To check the possibility that the hood may provide a mechanical stimulus, Oishi set two groups: the head was covered completely in birds of one group and a small hole was made in the eye region of the hood for the other group. Although the birds whose heads were covered completely showed testis regression, the birds whose hood had a small hole in the eye region maintained mature testis weight. However, Ivanova (1935) reported that the house sparrow (*Passer domesticus*) could receive light through

the body surface as well as the eyes. In her experiment, she covered the head with a silk hood with solid black blinkers fixed over the eyes. But she did not mention whether the silk hood was black and light-proof so as to prevent all light penetration. It seems possible that Ivanova did not consider the penetration of light into the extraretinal photoreceptor in the head, through the silk covering, but considered only the effect of light on the eyes. She also reported that there was a greater rate of recrudescence of the testes among nuded birds (the feathers of the back and breast were shaved) than among those which were fully feathered, and suggested that light might affect the gonads directly through the body surface. However, these two groups did not show a statistically significant difference in testis weight ($p > 0.1$), according to my recalculation of her data. Consequently, Ivanova's experiment does not seem to prove that light affects the gonads directly through the body surface, other than the head.

Experiment II showed the existence of an extraretinal, extrapineal photoreceptor in the maintenance of mature testes in adult Japanese quail. These results agree with those of Sayler and Wolfson (1968 b), who reported that neither the eyes nor the pineal are neces-

sary for initial development of the gonads in young Japanese quail. An extraretinal, extrapineal photoreceptor also seems to participate in light/dark cycle entrainment of oviposition in the chicken (Harrison and Becker, 1969) and in entrainment of locomotor activity in the house sparrow (Menaker, 1971). Experiment II also showed that the gonads responded to light even if the empty eye sockets were coated with opaque material, and even in the absence of the pineal body. These results suggest that light penetrates through the skull into some extraretinal, extrapineal photoreceptor elsewhere in the head. Benoit (1964) reported that direct light stimulation of the anterior hypothalamus or the rhinencephalon, via a quartz rod introduced across the eye socket of enucleated ducks, caused strong testicular growth. From these results, together with others demonstrating action spectrum differences in response (Benoit and Assenmacher, 1966; Benoit et al, 1966), Benoit concluded that the duck has a deep photoreceptor (hypothalamus and/or rhinencephalon) as well as superficial photoreceptors (eyes).

Wetterberg et al (1970 a, b) reported that the Harderian glands might be responsible for the light/dark cycle control of serotonin and HIOMT rhythms in the pineal glands of neonatal rats. The extraretinal photoreceptor

in neonatal rats which was reported by Snyder (1968) and Zweig et al (1966) can be interpreted on this basis. However, Wetterberg et al (1970 a) further attempted to explain Benoit's results on this basis. That is, they suggested that light which was introduced through a quartz rod might have stimulated the Harderian glands. But experiment III suggests that the Harderian gland is not necessary for the maintenance of mature testes in blinded and pinealectomized quail. It is also reported that the Harderian glands are not necessary for entrainment of diurnal behavior of the sparrow (Silver and Menaker, unpublished data quoted by Menaker, 1971).

Wetterberg et al's report (1970 a) on porphyrin pigments in the neonatal rat Harderian gland suggested that these might be the light-absorbing pigments responsible for the observed photoresponse. However, only the Harderian gland in rodents of the genus Mus exhibited a red fluorescence characteristic of porphyrin pigments, and several other animals examined (rabbit, guinea pig, chicken, magpie, turtle, frog) did not exhibit red fluorescence of this tissue (Derrien and Turchini, 1924, quoted by Grafflin, 1942; Klüver, 1944). The Harderian gland of Japanese quail likewise did not show red fluorescence when examined under ultraviolet light (Oishi, unpublished

observation). Consequently, any photosensitivity of the Harderian gland might be restricted to rodents of the genus *Mus*.

Homma (1969) reported that radioluminescent paint caused testis development in Japanese quail when it was put 1) in the orbital cavity between the eye muscles and the sphenoid bone, 2) at the base of the longitudinal fissure between the two cerebral hemispheres, 3) on the olfactory lobe or 4) in the thalamic or hypothalamic area.

A considerable amount of light is reported to pass into the brain through the skull in birds (Benoit, 1964) and in mammals (Klüver, 1944; Ganong *et al*, 1963; Van Brunt *et al*, 1964; Viggiani *et al*, 1970). Klüver (1944) also reported that the cortex of the brain and white matter exhibit a greenish fluorescence and a reddish fluorescence respectively, suggesting the existence of porphyrin or other pigments in the brain. He then extracted porphyrin pigments from each part of the brain. All of these reports strongly suggest that the brain itself may function as a photoreceptor.

In adult mammals, Lisk and Kannwischer (1964) reported that direct illumination of the hypothalamus via a glass rod caused changes in the estrous cycle, and in ovarian and pituitary weights. However, the eyes seem

to be the primary photoreceptors for the photoperiodic gonadal response (Critchlow, 1963; Wurtman, 1967; Moore, 1969). The report of Lisk and Kannwischer (1964) appears to be the only one suggesting an extra-retinal photoreceptor in adult mammals. Although sufficient light entered the brain under their experimental conditions, it remains to be established whether or not this occurs under natural conditions. If it does not occur, the phenomenon is not important physiologically, even though it is interesting phylogenetically.

The photoreceptive function of the eyes in eliciting the photoperiodic gonadal response in birds has been accepted for some time, but Menaker _et al_ (1970) recently reported that the eyes do not participate in photoreception for that response of the house sparrow, based on the finding that the gonads did not respond to light when India ink was injected under the skin of the head even if the eyes were intact. Homma (1969) reported the failure of radioluminescent paint, put in front of the retina, to induce the gonadal response of Japanese quail.

On the other hand, Benoit (1964) reported that light did bring about testicular growth when limited to the ocular region, the remaining parts of the head and

the body being shielded from light. When the eye socket, after enucleation, was covered with an opaque material (metal, rubber, black paraffin), the gonads did not respond to light. He also reported that the sensitivity to light intensity was about one to five lux in the normal duck, and 5 to 25 lux in the enucleated duck. The spectral sensitivity of the deep photo receptor extended across the entire visible spectrum whereas it was limited for the retina to red light. Benoit and his colleagues later showed that the action spectrum for the eye was between 625 nm and 647 nm, with peak responsiveness at 637 nm (Benoit and Assenmacher, 1966; Benoit _et al_, 1966). From these results, he concluded that the eye is one of the photoreceptors in the photoperiodic gonadal response. Ishibashi (1957) confirmed Benoit's experiment by showing that the testes of male chickens responded to light after enucleation but they did not respond when the eye sockets were coated with black gum. In Japanese quail, Oishi (1967) illuminated the eyes or the eye sockets (after removal of the eyeballs) through a small hole made in a head cover, and found that the testes responded to light, but he could not show an effect of light when the eye sockets were coated with black gum arabicum and illuminated through a hole in the hood. This suggests that light

stimulates gonadal activity through the eye or the region around the eye. He also showed that the gonads of enucleated birds responded to light when the whole head was illuminated except for the eye socket region which was covered with black gum (Oishi et al, 1966). This suggests that some part of the brain other than the eye region also participates in photoreception for maintenance of mature testis weight. There were slight differences between intact and enucleated birds in the response of the testes to several portions of the visible spectrum at low light intensity (see Section III-B). That is, green light at an intensity of 9.5 $\mu w/cm^2$ was not effective in blinded birds, while it was effective in intact ones. These results in the duck, the chicken and Japanese quail suggest that the eyes act as a photoreceptor or at least as a light guide for some deep photoreceptor.

The discrepancy between Homma's result and mine could be due to the low intensity of light in Homma's experiment, which might have been lower than the threshold to induce the gonadal response, or it could be due to the difference in the phase of the photoperiodic testicular response studied (growth rather than maintenance). The discrepancy between results with the house sparrow (Menaker et al, 1970), in which the eyes are not involved in photo-

reception for the photoperiodic gonadal response, and other species of birds (chicken, duck, and Japanese quail), in which the eyes do seem to be involved in photoreception, at least as a light guide, could represent species differences. It is interesting to note that the house sparrow shows photorefractoriness (a phenomenon in which gonads regress spontaneously after a certain period under long photoperiod), while the chicken and Japanese quail do not show such a phenomenon.

Bilateral superior cervical sympathectomy has been reported to delay the time of onset of oviposition in Japanese quail (McFarland _et al_, 1968; Sayler and Wolfson, 1968 b), although bilateral enucleation of the eyes did not have any effect on ovipositon (Sayler and Wolfson, 1968 b). It was also reported that the eyes are not necessary for the pinealectomy-caused ovarian maturation at precisely the "critical period" preceeding the onset of oviposition (Sayler and Wolfson, 1968 b). Hedlund _et al_ (1971) reported that bilateral sympathetic denervation prevented the light-induced diurnal serotonin peak in Japanese quail. Lauber _et al_ (1968) reported that neither bilateral enucleation of the eyes nor sympathetic denervation prevented the light-induced elevation of pineal enzyme activity (HIOMT) in chicks, and concluded

that in birds, in contrast to mammals, neither the retina nor sympathetic innervation of the pineal body is essential for environmental control of melatonin formation. In this connection, the observations by Morita (1966 b) and by Oksche et al (1969) are interesting. Both found that illumination of the pigeon's lateral eyes induced no electrical activity in the central part of the pineal body. The results described above suggest that the superior cervical sympathetic ganglion in birds does not seem to be the pathway for photic information from the retina to the pineal, in contrast to the situation in mammals (Wurtman et al, 1968; Taylor and Wilson, 1970; Schapiro and Salas, 1971). However, the information from light stimulation of the hypothetical deep photoreceptor may be transmitted through the ganglion to the pineal.

The above lines of evidence still do not resolve the question of whether the eyes of birds can be, or never are, involved as photoreceptors for the photoperiodic gonadal response, and/or whether there might be species differences.

Oishi and Kato (1968) provided evidence that the pineal body might be one of the photoreceptors for the photoperiodic gonadal response in Japanese quail. In their experiment, the pineal was illuminated locally by

putting radioluminescent paint on the top of the skull, in the region of the pineal. Paint emitting orange-red light caused maintenance of mature testis weight, but green-yellow light emitting paint did not support mature testis weight. Orange-red light was ineffective in pinealectomized animals. Munns (1970) covered the pineal region of the canary with a layer of black polyester resin, and found that testis weight was reduced to one third of control values. On the contrary, Homma (1969) failed to produce gonadal growth in young Japanese quail by local illumination of the pineal with the same kind of radioluminescent paint as described above. However, he seems to have used much smaller amounts of paint than that used in Oishi and Kato's experiment. I have shown that a large amount of paint is necessary to maintain gonadal activity (see Section III-B).

Morita (1966 b) and Oksche et al (1969) failed to show electrical activity in the pigeon pineal body in response to light. Ralph and Dawson (1968) also failed to show an electrical response of the pineal to illumination in Japanese quail and in the house sparrow.

An effect of light on pineal morphology and ultrastructure in birds was reported by Fujie (1968), by Basrur and Winget (1963) and by Quay and Renzoni (1963).

A "deformed" structure of the photoreceptors has been observed by several investigators (Oksche and Vaupel-Von Harnack, 1966; Fujie, 1968; Bischoff, 1969; Oksche et al, 1969; see also Section IV-D). Bischoff observed lamellar structures reminiscent of retinal cones in Japanese quail and chicken pineals, and suggested from this evidence a photoreceptive function of the avian pineal. On the contrary, Oksche and his colleagues (1966, 1969) and Collin (1971) concluded that the avian pineal is not a photoreceptor because of the degenerated appearance of the photoreceptor cell outer segment.

As in the case of the eye, the pineal has been considered by some investigators to serve in focussing light on a deep photoreceptor (Farner, 1970; Ralph, 1970, Menaker, 1971). Thus Ralph (1970) noted, "The pineal in most birds appears to be in an appropriate position to serve as a light funnel, conducting light from its expanded distal end just beneath the venous sinus and vault of the skull through its elongated stalk between the cerebrum and cerebellum to its insertion on the roof of the diencephalon. That is, photic information about the bird's environment may be preferentially conducted by the relatively transparent pineal to the diencephalon receptors". Since the discrepancy between the results

reported by Homma (1969) and by Oishi and Kato (1968) can be explained on the basis of the absorption of light by tissues overlying a deep photoreceptor, the pineal may be functioning as a light funnel as Ralph suggested. However, this discrepancy can also be explained on the basis of the threshold of photosensitivity of the pineal body, or the different phase of the photoperiodic gonadal response studied (Homma measured the response in growth of the gonads in young quail, while I measured the response in maintenance of mature gonads). A direct effect of light on the pineal body has been reported recently by Rosner <u>et al</u> (1971), which confirms the report by Lauber <u>et al</u> (1968). The former workers reported that explants of the duck pineal cultured in light showed a significantly higher melatonin synthesizing activity than those cultured in darkness. It remains to be examined whether light-controlled HIOMT activity in the pineal has any relation to gonadal activity, because the relation between HIOMT activity and gonadal activity is obscure (Ralph, 1970; see also Section IV-A and IV-B). However, the photoreceptive function of the pineal body seems to play, if anything, an auxiliary role in the photoperiodic gonadal response, because the gonadal response to light occurred in the absence of the eyes and the pineal. The pineal might

serve as the main or only photoreceptor in other systems such as the photoperiodic control of adrenal activity, since pinealectomy abolished the response of the pituitary and adrenal to the photoperiod in young Japanese quail, while it did not abolish the response of the gonads to the photoperiod (see Section IV-A).

B. EFFECTS OF LIGHT INTENSITY ON GONADAL MAINTENANCE, AND THE ACTION SPECTRUM OF THIS RESPONSE

INTRODUCTION

In this section, I concentrate on the effects of intensity and wavelength of light on gonadal responses, with special reference to the role of the extraretinal photoreceptor.

Many investigations have been concerned with the effects of light intensity on gonadal activity, especially with a view to improving egg production of domestic hens. In chickens, the threshold for maximum response in oviposition is reported to be in the range of 1 - 10 lux; further increase of light intensity above the threshold does not induce better egg production, nor lead to earlier onset of oviposition (Roberts and Carver, 1941; Dorminey et al, 1970). In the turkey, 2 ft. c. (21 lux) is claimed to be the minimum intensity to produce the maximum response in oviposition (Asmundson et al, 1946, 1951). For grouse and pheasants, the precise threshold has not been determined, but 22 ft. c. (235 lux) was sufficient to induce early onset of egg production, while sexual maturation was delayed under 0.02 ft. c. (0.21 lux) of light intensity (Clark et al, 1937). In the house sparrow, Bartholomew (1949) showed that the threshold for sper-

matogenesis is about 0.7 ft. c. (7.5 lux). He observed no difference in response to intensities higher than 10 ft. c. (108 lux). Farner (1959) also reported for the white crowned sparrow that the response of the testes was stronger at 31 lux than 11 lux, but that no further response occurred with higher light intensities. In the bobwhite quail, Kirkpatrick (1955) reported that light intensities in the range of 0.1 - 300 ft. c. (1 lux - 3,240 lux) showed the same effects on the testes or ovary and oviduct weight. He concluded that the threshold is less than 0.1 ft. c. (1 lux). In the duck, Benoit (1964) reported that the threshold of light intensity to induce gonadal development is 1 - 5 lux.

Only a few studies have been directed toward responses of the extraretinal photoreceptor to light intensity changes. Benoit (1964) reported that in ducks the threshold intensity for activating the extraretinal photoreceptor is 5 - 25 lux. In the house sparrow, Menaker and his colleagues reported that the threshold is less than 10 lux, and testis weight under several photoperiods and various intensities (20 - 500 lux) was the same in blinded as in intact sparrows (Menaker et al, 1970; Underwood and Menaker, 1970 a).

So far, in most of the species studied, the threshold intensity of light to induce gonadal development seems

to fall in the range of 1 - 20 lux, except in the study on European starlings by Bissonnette (1931 a, b), who reported that an increase of light intensity (up to 240 lux) produced a graded increase in spermatogenic response.

Several investigators have varied the wavelength of light used, in a search for the action spectrum of the photoperiodic gonadal response. Bissonnette (1932, in European starlings) used red, green or violet filters, transmitting intensities of 2.6 ft. c. (28.6 lux), 2.6 ft. c. or 0.32 ft. c. (3.52 lux), respectively. Initially, white light produced the greatest acceleration of testis activity, red next, while green light had a slight inhibitory effect. After prolonged treatment, birds under red light surpassed those receiving white light, and green light produced a definitely inhibitory effect. He concluded that long wavelengths are stimulatory and short ones inhibitory.

Several other investigators have also reported, for various species of birds, the stimulatory effect of long wavelengths and less stimulation, or inhibition, with shorter wavelengths. Burger (1943) used a series of Corning filters before a mercury vapor lamp or an incandescent lamp and found that only wavelengths between approximately 580 - 680 nm were able to stimulate starlings to produce sperm. Light intensity in these experiments

varied from 6.5 ft. c. (70 lux) to 100 ft. c. (1080 lux). For the duck, Benoit and his colleagues (Benoit and Assenmacher, 1966; Benoit et al, 1952, 1966) showed that wavelengths between 617 and 740 nm were effective at intensities of 81 ergs/cm^2/sec, and only wavelengths between 637 nm and 647 nm were effective at lower intensities (2.7 ergs/cm^2/sec). They postulated that at higher intensities the effect was on both the eyes and the deep photoreceptor (hypothalamus ?) and at lower intensities the effect was solely on the eyes. Hollwich and Tilgner (1961 a, b), in experiments employing monochromatic light (436, 546, 632 or 707 nm) at equal intensities of 245 μw/cm^2, also showed that the testis weight of ducks irradiated with 707 and 632 nm was many times higher than with shorter wavelengths. In the chicken, Dakan (1934) showed that red, yellow and white light were more effective for egg production than blue light. Ishibashi and Kato (1951) also reported that red light (570 - 680 nm) was stimulatory for testicular development while violet (400 - 490 nm) was ineffective (intensity was 5 - 30 lux in both cases). Platt (1953) showed that dim red light (15 or 10 watts, intensity of light at the bird's level was not reported) could maintain winter egg production of hens as well as white light. For testis development and initiation of

spermatogenesis, a difference between the effects of red light and blue or green light was reported by Harrison _et al_ (1969, 1970). Scott and Payne (1937) reported that turkey hens subjected to long photoperiods of white light (57 ft. c. = 616 lux) or long wavelengths (622 nm, 14 ft. c. = 151 lux) reached sexual maturity several months in advance of the normal breeding season, while short-wavelength light (425 nm, 1 ft. c. = 11 lux) did not influence age at sexual maturity. Ringoen (1942) showed greater testicular enlargement in English sparrows receiving continuous red light than in birds receiving continuous green light. In Japanese quail, Woodard _et al_ (1968) reported that male birds brooded under red light developed testes that were twice and three times heavier, respectively, than those of quail under green or blue light. Females maintained under red light reached a 50 per cent rate of egg production approximately 2 weeks earlier than those under blue or green light, and maintained higher egg production thereafter. The results of Aho _et al_ (1970) suggest that infrared light (2,300 nm or 3,000 nm) delays sexual maturity in both male and female quail.

The reports cited above support the stimulatory effect of red light and no effect or an inhibitory effect of far red, or of blue light. However, there are three

reports which showed the opposite effects or no influence of wavelength on gonadal activity. Rowan (1938) reported that several wavelengths (intensity 165 lux) induced uniform gonadal development in the sparrow and junco, but he did not specify which wavelengths, or give transmission curves of the filters used. Carson et al (1958) could not find any influence of wavelength on sexual maturity and egg production of chickens. For sperm production, red and gold light were non stimulatory (fluorescent light was used, and light intensity was at "similar" levels in the various light treatments). Carson suggested that the absence of an effect of blue light in other experiments could have been due to the small amount of light available to the birds, and that, at equivalent energy levels, blue light might also have hastened sexual maturity. They admitted, however, that the purity of colors used can sometimes be criticized (blue light in their experiments ranged between 400 - 600 nm, with a peak at about 440 nm). Schildmacher (1963) reported that a high intensity of blue light (150 or 200 lux, spectral width 380 - 480 nm) was effective in inducing gonadal growth in several species of passeriform birds, while only long wavelengths (but not infrared) were effective at low intensity (20 - 30 lux).

However, none of the investigations cited above

have clearly distinguished and controlled all four variables involved in photobiological experiments, that is, photoperiod, light energy, wavelength, and number of photons reaching the photoreceptor. Even in experiments in which energy levels are strictly equalized at several wavelengths, the corresponding number of photons is small in blue light and large in red light. So, a difference in response under the same intensity (energy) of light at different wavelengths could be due to the difference in the number of photons provided or received. Intensity measured in luminosity (lux) does not indicate the amount of energy or the number of photons. The experiments reported here were designed to check the importance of these variables in interpretation of one photobiological effect, the photoperiodic testicular response of Japanese quail.

In the previous section (Section III-A), I reported that an extraretinal photoreceptor has an important role in the photoperiodic gonadal response. There are two reports which deal with the action spectrum and location of the extraretinal photoreceptor for the photoperiodic gonadal response. Benoit and his colleagues reported that, when light was introduced directly to the hypothalamus with a quartz rod, long wavelengths (red) as well as intermediate (yellow) and short wavelengths (indigo and blue) were effective in stimulating testicular

growth (Benoit, 1964). The amount of light penetration into the brain at each wavelength was measured by either a photographic or a photoelectric method. Transmittance through the tissues was very weak for the short wavelengths (1/2,000 of the applied intensity for indigo and 1/630 for green), but appeared to be greater for longer wavelengths (1/130 for orange and 1/55 for red). Consequently, Benoit concluded that red light can penetrate to the hypothalamus with sufficient intensity to activate the deep photoreceptor, adding its effect to that via the retina. Homma (1969) confirmed Benoit's results, showing that radioluminescent paint, implanted in the brain, emitting either orange-yellow light (575 nm) or blue light (455 nm) induced a gonadal response in Japanese quail. However, these reports showed the effects of light directly introduced to the presumed extraretinal photoreceptor. I here report an attempt to determine the action spectrum for the photoperiodic testicular response (maintenance of mature testes) in blinded birds.

MATERIALS AND METHODS

Experiment I - Effect of light intensity on the maintenance of mature testes of intact and enucleated birds: The experimental design is shown in Figure 8. Ten and 12 week old birds, which were reared under a photo-

period of 14 hours light per day (14L/10D) for 4 or 6 weeks after hatching and under continuous light (24L/0D) for the following 6 weeks, were divided into 7 groups. Birds of groups A, C, and E were intact, and in birds of B, D, F, and G groups the eyes were enucleated. Unilateral enucleation was done 4 days before the beginning of the experiment and the second eye was enucleated 2 days later. The birds in groups A and B received light of intensity 15.7 $\mu w/cm^2$ (26 lux, here called x 1 dim light, measured at head level in the center of the chamber). The birds in groups C and D received 1.57 $\mu w/cm^2$ (2.6 lux, here called x 0.1 dim light), and birds in groups E and F received 0.157 $\mu w/cm^2$ (0.26 lux, here called x 0.01 dim light). Group G birds were kept in continuous darkness (0L/24D). A single 7.5 W incandescent light bulb mounted in the top of the environment chamber constituted the light source. Sheets of glass sprayed lightly with black paint were used as neutral density filters to reduce the light intensity. Temperature was 29.9 ± 0.4 °C. After 3 weeks under these light intensity conditions, all birds were killed and body weight and testis weight were recorded.

Experiment II - Effect of wavelength on the maintenance of mature testes of intact birds: The experimental design is shown in Figure 10. Birds (11.5 and 13.5 week old), which were reared under continuous light, were divided into 10 groups. A, B, C, and D groups were main-

tained under 24L/0D at x 1 dim light. E, F, G, H, and I groups received x 0.1 dim light under 24L/0D. The eyes of group F birds were enucleated. Group J birds were kept in continuous darkness (0L/24D). A, E, and F birds were maintained under white light. B and G groups were exposed to red light, C and H groups were in green light, and D and I groups were in blue light. Spectral environments were achieved by placing colored plastic filters* in front of an incandescent light source (150 W photoflood**). Cupric sulfate (10% solution, 3 cm deep) before the filter was used to remove excess heat. To adjust the light intensities, neutral density filters were used. Transmitted light energies were as shown in Figure 7. The values for measured energy and calculated luminous intensity and number of photons are shown in Table IV. Temperature was 27.9 ± 0.3 °C. After 4 weeks of the experiment, all birds were killed and body weight and testis weight were recorded.

Experiment III - Effect of wavelength on the maintenance of mature testes of intact and enucleated birds: The experimental design is shown in Figure 12. Eleven and 13 week old birds, which were reared in 14L/10D for 2 weeks after hatching and in 24L/0D thereafter until the beginning

---

* Carolina Biological Supply House for red and blue filter, Rohm and Hass Plexiglas for green filter.

** Sylvania PAR 38, Sylvania Electric Co., Montreal, Quebec

of the experiment, were divided into 10 groups. A, B, C, D, and E groups were intact animals, and those of groups F, G, H, I, and J were enucleated. A and F groups were kept under white light, B and G groups were in red light, C and H groups in green light, and D and I groups in blue light. All of the groups above were maintained under 23L/1D during the experiment. The spectral energies were the same as x 1 dim light in Experiment II. Group E birds were kept in 1L/23D and group J birds were kept in 0L/24D as controls. The temperature was 27.5 ± 0.3 °C. After 3 weeks of the experiment, all birds were killed and body weight and testis weight were recorded.

Experiment IV - Effect of a small amount of radio-luminescent paint placed in the region of the pineal: The experimental design is shown in Figure 14. Eight week old birds reared under continuous light were divided into 5 groups. Group A and B birds served as controls. In group C, orange-red radioluminescent paint (ATOMLOIHI-P*, maximum light emitted at 600 nm) was placed on the surface of the brain immediately over the pineal body through a hole drilled in the skull. Black plastic tape was placed on the skull over the painted area, and the skin was sutured. The amount of radioluminescent paint for group C was approximately 5 mg

---

* Dai Nippon Sinloihi Co., Tokyo

per bird, mixed with binder. Group D birds received 0.5 mg of orange-red paint, and 8 mg of green-yellow paint (maximum emission at 520 nm) was used for group E. The photoperiod was 24L/0D for group A and 8L/16D for B, C, D and E groups. Thus, if the radioluminescent paint is capable of stimulating the photoreceptor for the photoperiodic gonadal response, the testes of short day birds in groups C, D and E would be expected to remain large as in group A birds. Temperature was 28.3 ± 0.2 °C. After 17 days of the experiment, all birds were killed and body weight and testis weights were recorded.

Experiment V - Effect of a larger amount of radioluminescent paint placed in the region of the pineal: The experimental design is shown in Figure 15. Six week old birds reared under 24L/0D were divided into 5 groups. F and G group birds served as controls. Orange-red radioluminescent paint (15 mg) was applied to the pineal area in group H birds, as in Experiment IV, and 15 mg of green-yellow paint was applied to group J birds. I group birds were pinealectomized one day before the start of the experiment, and orange-red radioluminescent paint (15 mg) was placed where the pineal had been. Group F birds were maintained under 24L/0D, and G, H, I, and J groups were kept in a short photoperiod (8L/16D) for 18 days. The temperature was 28.3 ± 0.2 °C. Body weight and testis weight were recorded at the end of the experiment.

RESULTS

Experiment I - Effect of light intensity on the maintenance of mature testes of intact and enucleated birds: The results are shown in Table V and Figure 9. Both group A (intact) and B (enucleated) birds maintained their testis weight under x 1 dim light. Birds of groups C (intact) and D (enucleated) under x 0.1 dim light showed considerably reduced testis weights in comparison with groups A and B quail ($p < 0.01$ and $p < 0.001$, respectively) and were not different from the dark controls (0L/24D, group G). Group E (intact) and group F (enucleated) birds, maintained under x 0.01 dim light also had greatly reduced testis weights (A : E $p < 0.001$; B : F $p < 0.05$). In x 0.1 dim light, the intact group C animals had larger testis weights than the enucleated group D birds ($p < 0.02$). However, this difference does not seem to be meaningful, because the enucleated birds in x 0.01 dim light (group F) and in total darkness (group G) showed higher values for testis weight than group D, although not significantly so, nor were these values significantly lower than for group C. Body weights of blinded animals and of those in continuous darkness were significantly smaller than for intact animals. This may have been because the birds were not sufficiently trained before the operation to find food in darkness. However, the above results are the same, whether the data are expressed as

absolute gonadal weights or gonado-somatic index (T. W./B. W.).

Experiment II - Effect of wavelength on the maintenance of mature testes of intact birds: The results are shown in Table VI and Figure 11. Under x 1 dim light, the birds in white light (group A), red light (group B), and green light (group C) maintained large testis weights, while the birds in blue light (group D) had greatly reduced testis weights ($p < 0.001$). Under x 0.1 dim light, the group E intact birds and group F enucleated birds in white light, and group G birds in red light maintained large testis weights, while the birds in blue light (group I) had significantly reduced testis weights, as did the group J controls in total darkness. Group H quail (x 0.1 green dim light) had testis weights intermediate between those of group G (red) and group I (blue). It should be noted that the testes of birds under green light responded differently to x 1 dim and x 0.1 dim light. That is, x 1 dim light was effective, but x 0.1 dim light was not, in maintaining testis weights.

Experiment III - Effect of wavelength on the maintenance of mature testes of intact and enucleated birds: The results are shown in Table VII and Figure 13. The intact birds under x 1 dim white (group A), red (group B) and green (group C) light maintained mature testis weights while the

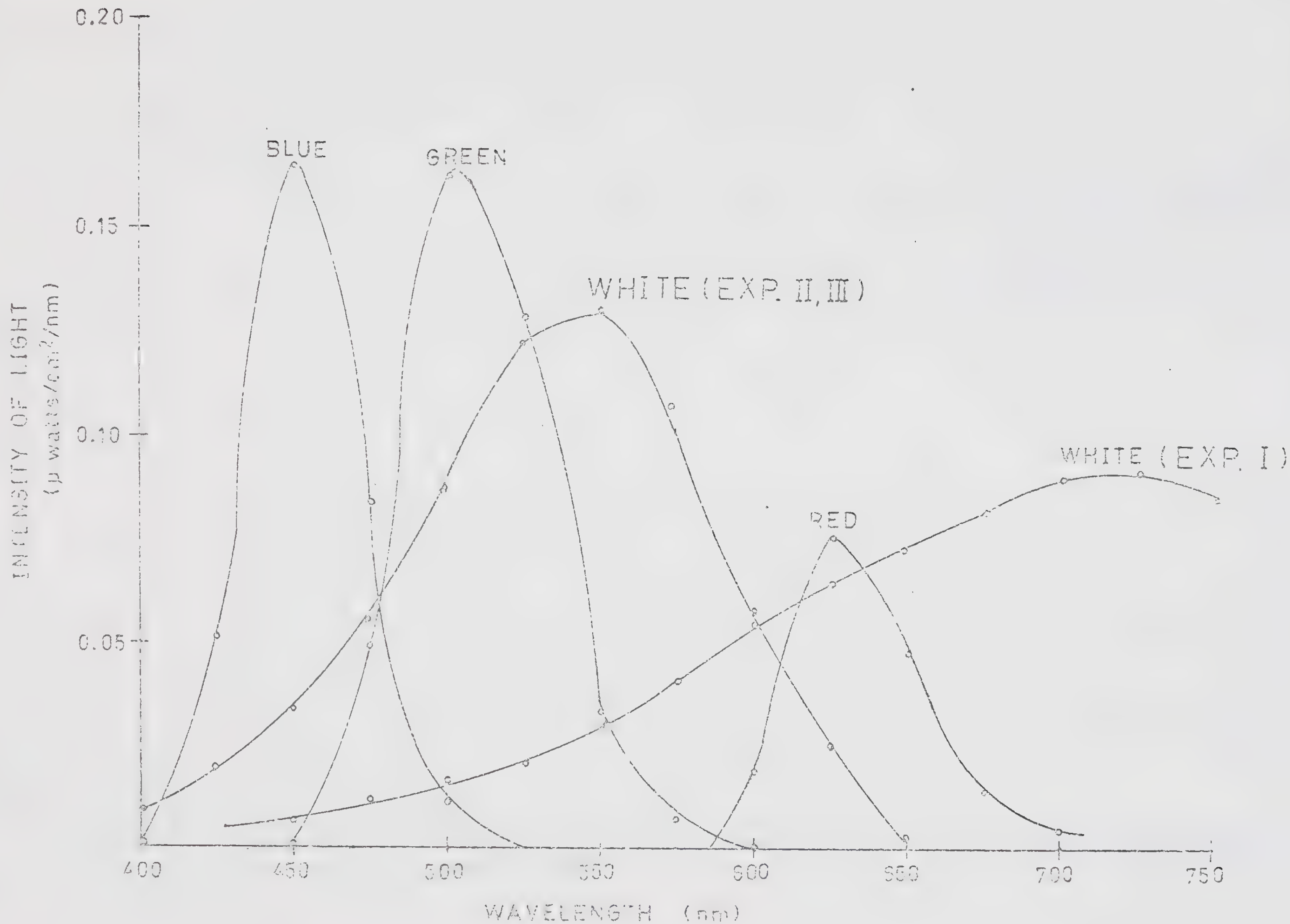
birds in blue light (group D) had testis weights reduced ($p < 0.001$) to control (group E) values. This confirmed the results of Experiment II. The enucleated birds in x 1 white dim light (group F) and red dim light (group G) maintained large testes, while the enucleated birds in green light (group H) and blue light (group I) showed considerably reduced testis weights ($p < 0.05$, $p < 0.01$, respectively). The results for the group I birds (blue) did not differ from the dark control birds (group J). The testis weights of group H (green light) quail were intermediate between those of birds under red and blue light, although they were not significantly smaller than those of group C (intact birds under x 1 dim green light). The same tendency was shown by the intact birds under x 0.1 dim light in Experiment II, ie, large testes in the red light birds, intermediate testes in green light birds, and small testes in blue light birds. Body weights of the enucleated birds were smaller than those of intact birds, as was observed in Experiment I. However, the above results are the same, whether the data are expressed as absolute gonadal weights or as gonado-somatic index (T. W./B. W.).

Experiment IV - Effect of radioluminescent paint placed in the region of the pineal: The results are shown in Table VIII and Figure 16. Group A birds (24L/0D, control) maintained large testis weights, while group B birds

(8L/16D, control) showed a significant reduction of testis weight ($p < 0.001$). All of the quail which had radioluminescent paint in the region of the pineal (group C - 5 mg orange-red paint; group D - 0.5 mg orange-red paint; group E - 8 mg green-yellow paint) failed to maintain large testes in the short environmental photoperiod ($p < 0.001$). Thus, neither orange-red nor green-yellow radioluminescent paint was stimulatory in the low concentrations used.

Experiment V - Effect of radioluminescent paint placed in the region of the pineal (higher intensity): The results are shown in Table VIII and Figure 16. The stimulatory effect of continuous light was again evident in group F (24L/0D) as compared to group G (8L/16D) ($p < 0.001$). Group H birds, to whose heads 15 mg of orange-red paint was applied, had average testis weights of 820 ± 124 mg, which is lower than that of group F ($p < 0.01$) but higher than that of group G (8L/16D) ($p < 0.05$) or of group J (8L/16D and 15 mg of green-yellow paint) ($p < 0.01$). The testis weights of group I birds (pinealectomized and with 15 mg of orange-red radioluminescent paint applied) were not statistically higher than those of group G and J birds, nor significantly lower than those of group H quail. Thus, in Experiment V, a higher intensity of light, emitted by a larger amount of radioluminescent paint than was used in Experiment IV, was capable of eliciting a photoperiodic

Figure 7. Spectral distribution and light energy under the filter systems used in Experiments I, II and III in Section III-B.



INTENSITY OF LIGHT
(μ watts/cm²/nm)
0.20
0.15
0.10
0.05
BLUE
GREEN
WHITE (EXP. II, III)
WHITE (EXP. I)
RED
400
450
500
550
600
650
700
750
WAVELENGTH (nm)

Table IV

Energy (measured by spectroradiometer) and calculated number of photons and luminous intensity at x 1 dim light

	Filter	Peak wavelength (nm)	Total intensity of light transmitted: Energy (μ w/cm^2)	Photons/cm^2/sec x 10^{13}	Lux
Experiment I					
White	N. D.*	725	15.7	3.9	26.0
Experiment II, III					
White	N. D.* + $CuSO_4$	550	16.7	4.5	71.0
Red	CBS** + $CuSO_4$	625	4.0	1.2	7.4
Green	R.H.P.*** + $CuSO_4$	500	9.6	2.5	33.0
Blue	CBS + $CuSO_4$	450	7.8	1.8	3.4

* Neutral Density Filter
** Carolina Biological Supply Filters
*** Rohm and Hass Plexiglas Filter
Source of light: 7.5 Watt incandescent light bulb for Experiment I, 150 Watt Sylvania PAR 38 lamp for Experiments II and III
Heat absorber: 10% $CuSO_4$ solution, 3 cm deep

Figure 8. Design of Experiment I (Section III-B) to test the effect of light intensity on the maintenance of mature testes of intact and enucleated birds.

x 1 dim light: 15.7 $\mu w/cm^2$ (26 lux)

x 0.1 dim light: 1.57 $\mu w/cm^2$ (2.6 lux)

x 0.01 dim light: 0.157 $\mu w/cm^2$ (0.26 lux)

Figure 9. Effect of light intensity on the maintenance of mature testes of intact and enucleated birds (Results of Experiment I in Section III-B). The vertical bar represents standard error.

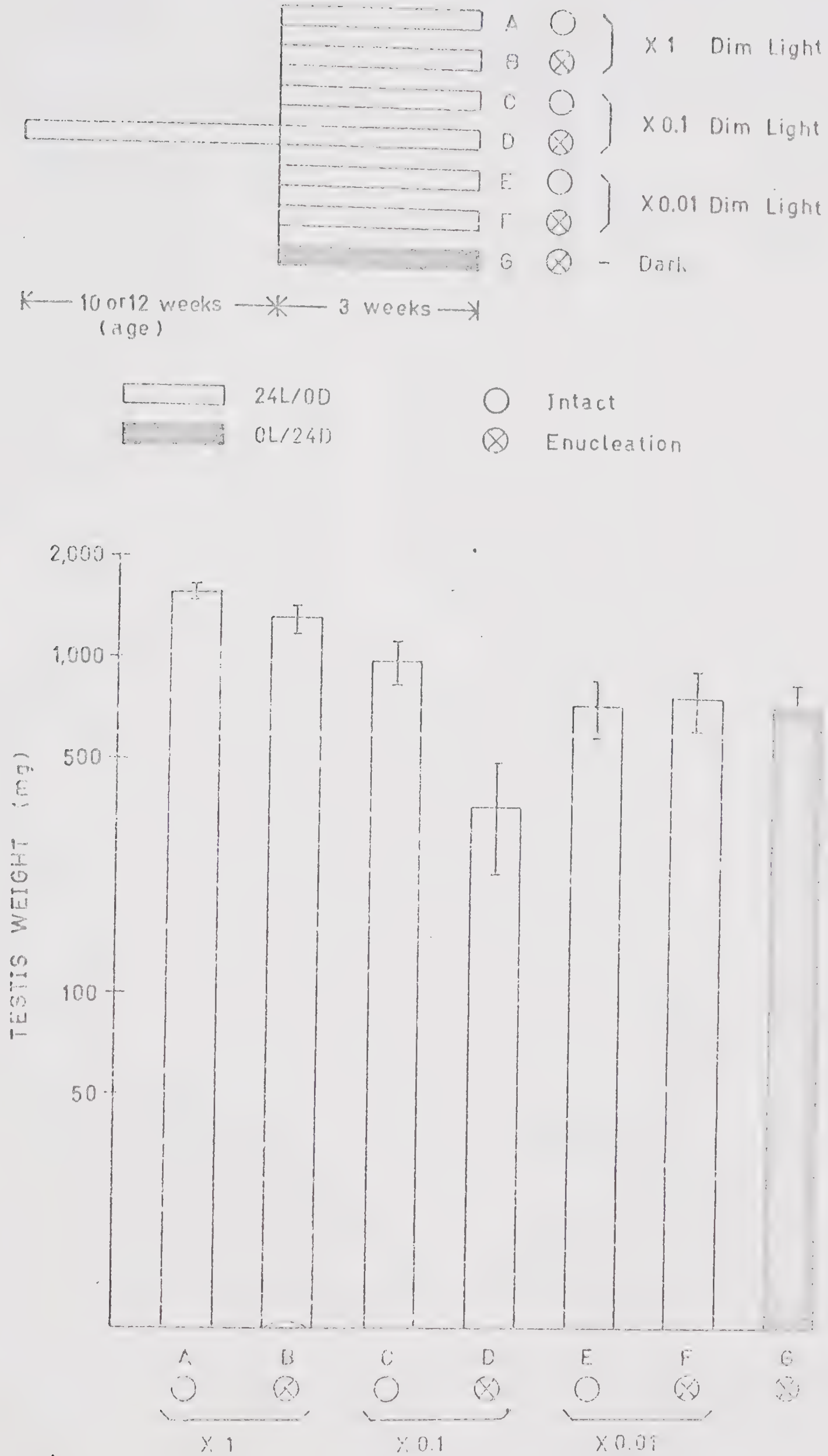

A
B
C
D
E
F
G
X 1 Dim Light
X 0.1 Dim Light
X 0.01 Dim Light
Dark
10 or 12 weeks
(age)
3 weeks
24L/0D
0L/24D
Intact
Enucleation
TESTIS WEIGHT (mg)
2,000
1,000
500
100
50
A
B
C
D
E
F
G
X 1
X 0.1
X 0.01

Table V

Effect of light intensity on the maintenance of mature testes of intact and enucleated quail (Results of Experiment I in Section III-B)

Group	Photoperiod	Number of Birds	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	G. S. I.
x 1 dim light					
A : Intact	24L/0D	14	117.8 ± 3.5	1,571 ± 77	1,346 ± 73
B : Enucleated	24L/0D	12	94.8 ± 1.8	1,307 ± 111	1,371 ± 108
x 0.1 dim light					
C : Intact	24L/0D	14	110.8 ± 3.0	991 ± 155	908 ± 144
D : Enucleated	24L/0D	10	88.2 ± 4.1	364 ± 139	396 ± 147
x 0.01 dim light					
E : Intact	24L/0D	14	110.9 ± 3.1	741 ± 155	685 ± 147
F : Enucleated	24L/0D	13	93.6 ± 2.9	794 ± 166	833 ± 164
Dark Control					
G :	0L/24D	14	88.9 ± 2.1	747 ± 117	846 ± 140

Table V - continued

Group	Photoperiod	Number of Birds	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	G. S. I.
Start					
H : Intact	24L/0D	8	102.6 ± 2.8	1,723 ± 132	1,671 ± 97
I : Enucleated	24L/0D	7	87.1 ± 3.4	1,345 ± 73	1,547 ± 70

Testis Weight:

A : C $p < 0.01$; A : E $p < 0.001$; A : G $p < 0.001$; B : D $p < 0.001$

B : F $p < 0.05$; B : G $p < 0.01$; C : D $p < 0.02$; H : I $p < 0.05$

G. S. I. (gonado-somatic index, mg T. W. x 100/gm B. W.)

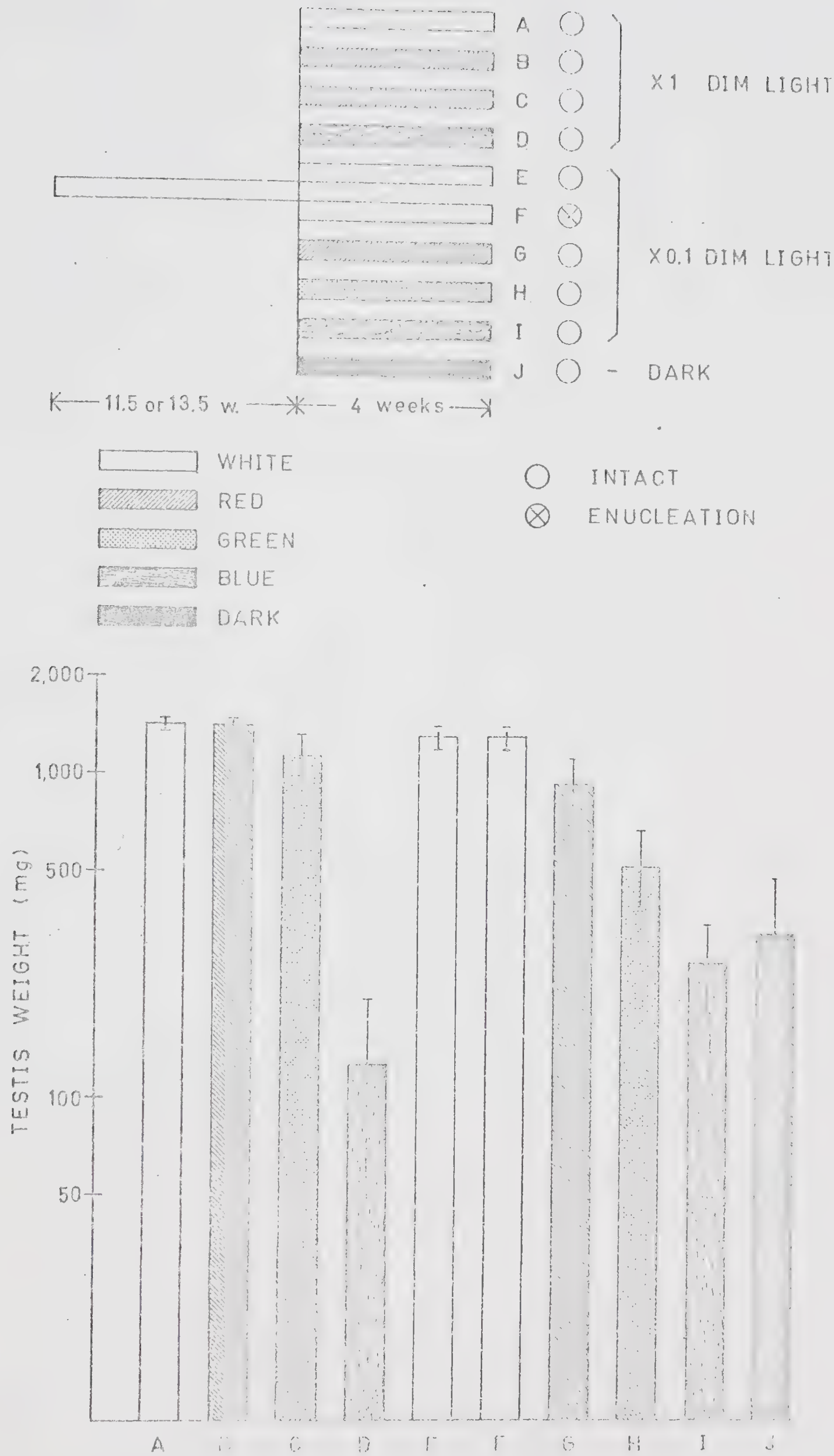
A : C $p < 0.02$; A : E $p < 0.01$; A : G $p < 0.01$; B : D $p < 0.001$;

B : F $p < 0.02$; B : G $p < 0.02$; C : D $p < 0.05$

Figure 10. Design of Experiment II (Section III-B) to determine the action spectrum for the maintenance of mature testes of intact birds at different light intensities.

Figure 11. Effect of wavelength on the maintenance of mature testes of intact birds at different light intensities (Results of Experiment II in Section III-B).

The vertical bar represents standard error.



A
B
C
D
E
F
G
H
I
J
X1 DIM LIGHT
X0.1 DIM LIGHT
DARK
11.5 or 13.5 w.
4 weeks
WHITE
RED
GREEN
BLUE
DARK
INTACT
ENUCLEATION
TESTIS WEIGHT (mg)
2,000
1,000
500
100
50

Table VI

Effect of wavelength on the maintenance of mature testes of intact quail

(Results of Experiment II in Section III-B)

Group	Photoperiod	Number of Birds	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	G. S. I.
x 1 dim light					
A : White	24L/0D	10	130.4 ± 4.0	1,403 ± 70	1,078 ± 50
B : Red	24L/0D	10	128.4 ± 3.7	1,410 ± 85	1,099 ± 59
C : Green	24L/0D	10	129.2 ± 4.0	1,152 ± 175	913 ± 146
D : Blue	24L/0D	9	122.9 ± 3.9	128 ± 73	109 ± 66
x 0.1 dim light					
E : White	24L/0D	10	125.3 ± 3.7	1,310 ± 109	1,047 ± 83
F : White (Enucleated)	24L/0D	10	116.9 ± 2.3	1,311 ± 104	1,121 ± 86
G : Red	24L/0D	10	125.0 ± 3.5	954 ± 190	774 ± 159
H : Green	24L/0D	10	122.7 ± 3.0	530 ± 157	444 ± 136
I : Blue	24L/0D	10	125.8 ± 3.5	267 ± 83	227 ± 80

Table VI - continued

Group	Photoperiod	Number of Birds	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	G. S. I.
Dark Control					
J	0L/24D	9	123.1 ± 3.5	324 ± 167	262 ± 134

Testis Weight:

A, B, C : D $p < 0.001$; C : H $p < 0.05$; E : G n. s.; E : H $p < 0.01$;

E : I $p < 0.001$; E : J $p < 0.01$; G : I $p < 0.01$

G. S. I. (gonado-somatic index)

p values are the same as those for testis weight.

Figure 12. Design of Experiment III (Section III-B) to determine the action spectrum for the maintenance of mature testes of intact and enucleated birds.

Figure 13. Effect of wavelength on the maintenance of mature testes of intact and enucleated birds (Results of Experiment III in Section III-B). The vertical bar represents standard error.

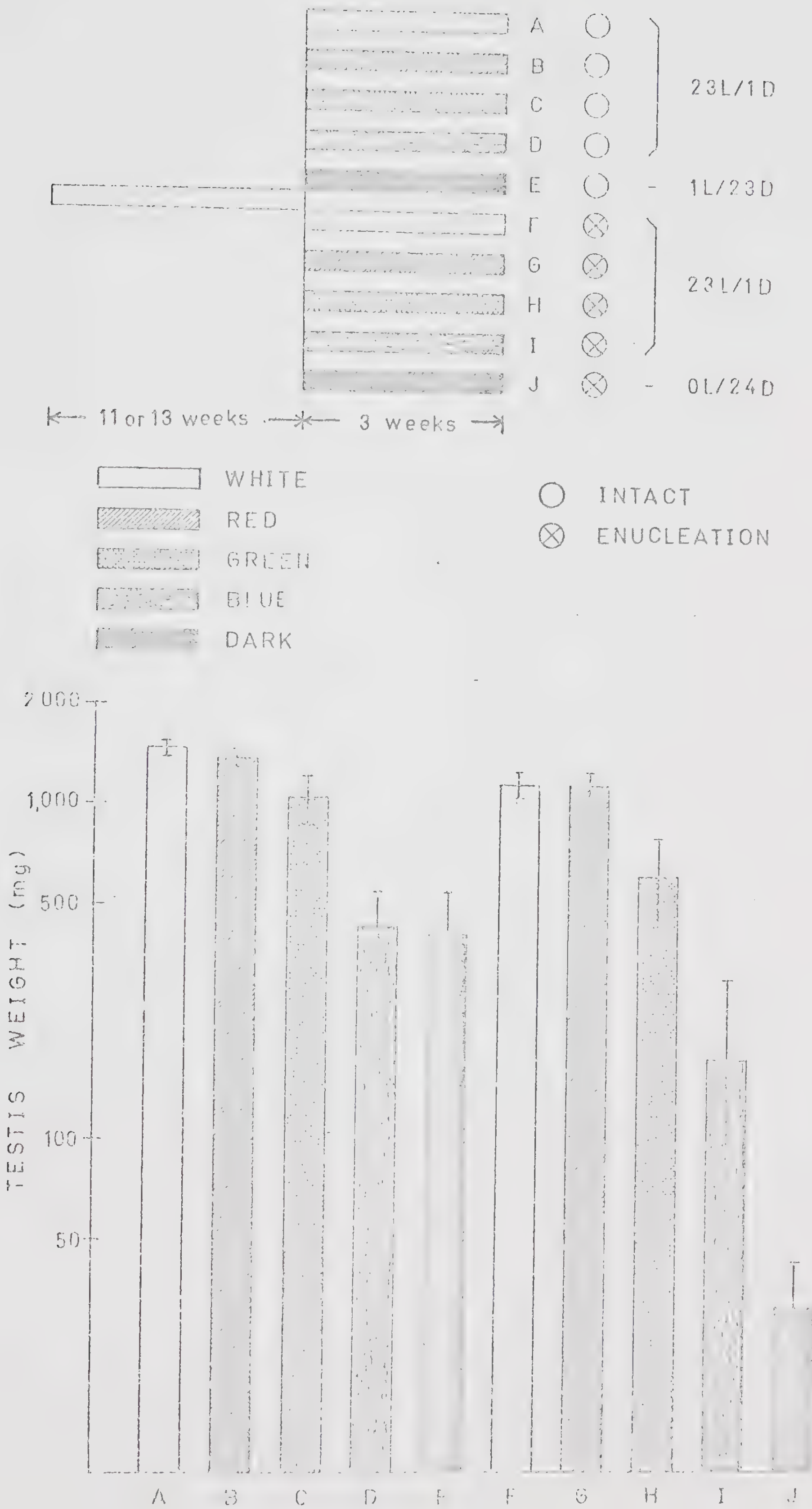

A
B
C
D
E
F
G
H
I
J
23L/1D
1L/23D
23L/1D
0L/24D
11 or 13 weeks
3 weeks
WHITE
RED
GREEN
BLUE
DARK
INTACT
ENUCLEATION
TESTIS WEIGHT (mg)
2,000
1,000
500
100
50

Table VII

Effect of wavelength on the maintenance of mature testes of intact and enucleated quail (Results of Experiment III in Section III-B)

Group	Photoperiod	Number of Birds	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	G. S. I.
Intact Birds					
A : White	23L/1D	9	120.1 ± 2.6	1,444 ± 84	1,201 ± 70
B : Red	23L/1D	9	114.8 ± 4.4	1,356 ± 76	1,200 ± 95
C : Green	23L/1D	10	113.4 ± 2.9	1,033 ± 178	928 ± 165
D : Blue	23L/1D	10	114.4 ± 4.1	428 ± 128	382 ± 119
E : Dark	1L/23D	10	101.6 ± 4.9	409 ± 140	397 ± 137
Enucleated Birds					
F : White	23L/1D	9	96.3 ± 2.0	1,152 ± 102	1,200 ± 109
G : Red	23L/1D	5	105.8 ± 4.1	1,151 ± 103	1,087 ± 90
H : Green	23L/1D	7	97.0 ± 2.6	612 ± 182	631 ± 184

Table VII - continued

Group	Photoperiod	Number of Birds	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	G. S. I.
Enucleated Birds					
I : Blue	23L/1D	6	100.1 ± 1.6	173 ± 129	175 ± 132
J : Dark	0L/24D	8	102.3 ± 2.5	31 ± 12	32 ± 14

Testis Weight:

A : D, E $p < 0.001$; C : D $p < 0.05$; F : J $p < 0.001$; G : H $p < 0.05$

G : I $p < 0.01$; H : J $p < 0.02$; E : J $p < 0.05$

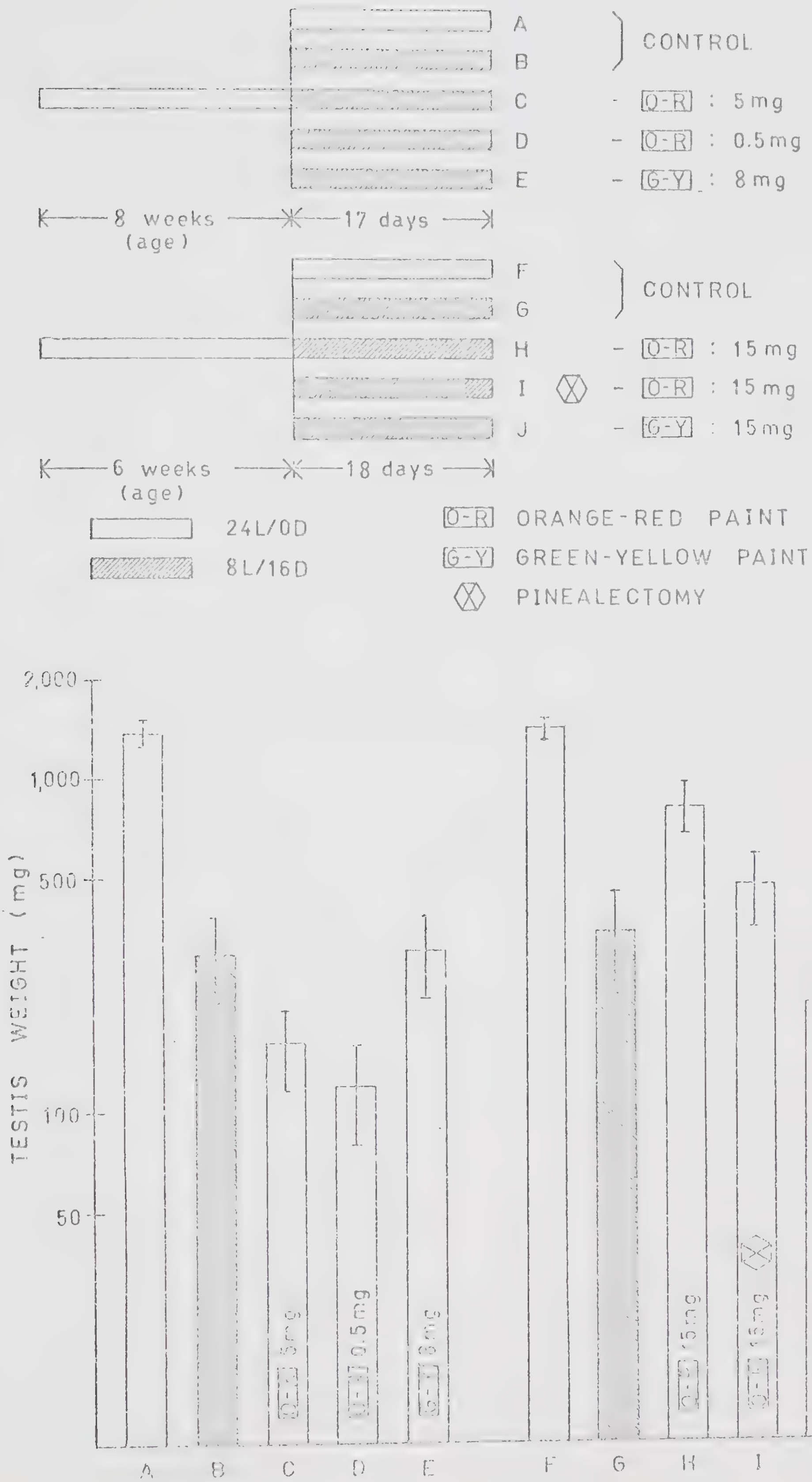
G. S. I. (gonado-somatic index)

p values are the same as those for testis weight.

Figure 14. Design of Experiment IV (Section III-B) to determine the effect of radioluminescent paint placed in the region of the pineal.

Figure 15. Design of Experiment V (Section III-B) to determine the effect of radioluminescent paint placed in the region of the pineal.

Figure 16. Effect of radioluminescent paint placed in the region of the pineal (Results of Experiment IV and V in Section III-B). The vertical bar represents standard error.



A
B
CONTROL
C
- O-R : 5mg
D
- O-R : 0.5mg
E
- G-Y : 8mg
8 weeks (age)
17 days
F
G
CONTROL
H
- O-R : 15mg
I
- O-R : 15mg
J
- G-Y : 15mg
6 weeks (age)
18 days
24L/0D
8L/16D
O-R ORANGE-RED PAINT
G-Y GREEN-YELLOW PAINT
PINEALECTOMY
TESTIS WEIGHT (mg)
2,000
1,000
500
100
50
O-R 5mg
O-R 0.5mg
G-Y 8mg
O-R 15mg
O-R 15mg
G-Y 15mg
A
B
C
D
E
F
G
H
I
J

Table VIII

Effect of radioluminescent paint placed in the region of the pineal body

(Results of Experiment IV and V in Section III-B)

Group	Photoperiod	Number of Birds	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	G. S. I.
Experiment IV					
A : Control	24L/0D	10	110.5 ± 3.4	1,378 ± 138	1,246 ± 131
B : Control	8L/16D	9	103.0 ± 3.6	298 ± 87	287 ± 83
C : 5 mg O-R paint*	8L/16D	10	100.4 ± 3.0	160 ± 46	158 ± 46
D : 0.5 mg O-R paint	8L/16D	9	103.8 ± 2.7	119 ± 40	116 ± 39
E : 8 mg G-Y paint**	8L/16D	9	103.3 ± 3.2	302 ± 86	302 ± 86
Experiment V					
F : Control	24L/0D	10	117.3 ± 3.9	1,436 ± 70	1,226 ± 49
G : Control	8L/16D	10	101.9 ± 3.3	348 ± 124	345 ± 119

* orange-red radioluminescent paint

** green-yellow radioluminescent paint

Table VIII - continued

Group	Photoperiod	Number of Birds	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	G. S. I.
Experiment V					
H : 15 mg O-R paint	8L/16D	11	103.2 ± 2.5	820 ± 162	792 ± 157
I : 15 mg O-R paint (Pinealectomized)	8L/16D	12	97.5 ± 2.1	479 ± 122	484 ± 114
J : 15 mg G-Y paint	8L/16D	10	104.6 ± 2.5	210 ± 93	205 ± 91

Testis Weight:

A : B, C, D, E $p < 0.001$

F : G, I, J $p < 0.001$; F : H $p < 0.01$; G : H $p < 0.05$; H : J $p < 0.01$

G. S. I. (gonado-somatic index)

p values are the same as those for testis weight.

gonadal response when the spectral emission was orange-red, but not when the light was yellow-green. However, the response was not as pronounced as that of birds kept under continuous light, probably due to the low intensity of light used.

DISCUSSION

Experiment I showed that the threshold of white light for maintenance of gonadal maturity in male Japanese quail is between 15.7 $\mu w/cm^2$ (26 lux) and 1.57 $\mu w/cm^2$ (2.6 lux) in both intact and blinded animals. However, in Experiment II, testis weight was maintained by light energy at the level of 1.67 $\mu w/cm^2$ (7.1 lux). Consequently, the threshold for the maintenance of mature testes seems to be around the intensity of 1.57 - 1.67 $\mu w/cm^2$ (2.6 - 7.1 lux), although the difference of the spectral distribution of white light between Experiment I and II (see Figure 7) has to be considered also. This value is in agreement with the value reported by several other investigators, studying the gonadal growth phase, as cited in the Introduction. Blinded birds in my experiments also responded to 1.67 $\mu w/cm^2$ light energy. Menaker _et al_ (1970) reported that the house sparrow's threshold of light intensity for extraretinal photoreception in the photoperiodic gonadal response (growth phase) is less than 10 lux. My

experiments confirm that even at low intensity the eyes are apparently not necessary for photoreception in the photoperiodic gonadal response.

Above the threshold, there was no further increase of response with an increase of intensity, although there is some suggestion of a graded response in a range near the threshold, which could be due to slight individual threshold differences. Burger (1939) reported that neither high nor low intensity of light was able to induce spermatogenesis in male starlings kept in a short photoperiod (10.5L/13.5D). This, together with several other reports (Kirkpatrick, 1955; Wilson et al, 1956; Hamner, 1963; Follett and Sharp, 1969) suggests that a sufficiently long photoperiod or the timing of the light period is a more important factor for birds than the intensity of light, after the threshold is attained. In mammals, on the contrary, several reports indicate that light intensity plays an important role in the photoperiodic gonadal response. Bissonnette (1935) and Marshall (1940), working with ferrets, showed that the degree of acceleration in the time of estrus was roughly correlated with the intensity of light. Marshall and Bowden (1936) reported that, when two ferrets were exposed to an intensity of 116 erg/cm^2 for a 2L/22D photoperiod and two other ferrets were exposed to 14

erg/cm^2 for 16L/8D (both pairs of animals thus received the same total quantity of radiation per day), there was little difference in the behavior of the animals and both pairs showed an acceleration of estrus. Williams (1969) reported that for mice, light intensity alone can influence age at first estrus. Recently, Vriend (personal communication) showed that numbers of photons, rather than the light energy, control gonadal activity in mice. If this is the case, a different cue is used by mammals than by birds, for the photoperiodic gonadal response. It is interesting to note that the eyes are the primary photoreceptor in mammals, whereas some part of the brain is responsible for the photoperiodic gonadal response in birds. This difference in the site of the photoreceptor might be the reason for the different responsiveness, although differences in CNS integration of the light information could also be involved.

In Experiment II, intact animals were stimulated by x 1 dim red and green light (4.00 and 9.6 μ w/cm^2, respectively), but only red light was effective a x 0.1 dim light (0.4 μ w/cm^2). Experiment III showed that x 1 dim green light (9.6 μ w/cm^2) was not stimulatory to enucleated birds.

Seven factors (wavelength, number of photons, energy of light, spectral transmittance of filters, photoperiod, the location of the photoreceptor, and the absorp-

tion of light by tissues) have to be considered in discussion of the above results. In intact birds, red and green light were effective and blue light was ineffective at low intensity (x 1 dim light) and only red light was effective under very low intensity (x 0.1 dim light) conditions. Since the green filter transmitted a range of wavelengths between 450 nm and 550 nm with a maximum at 500 nm (see Figure 7), effective long wavelength light sufficient to maintain gonadal activity might have penetrated through the green filter at the higher intensity. At the lower intensity the effective (threshold) amount of light might have been cut off by the green filter so as to yield results intermediate between those of red and blue light. Blue light presumably could not maintain gonadal activity because the blue filter (range of wavelengths 400 - 500 nm) completely cut off the effective wavelengths. However, the number of photons differs depending upon the wavelength when the same amount of energy is used (red > green > blue), whereas the amount of energy differs depending upon the wavelength when the same numbers of photons are used (blue > green > red). My experiments suggest that neither energy level nor number of photons is the key variable in the bird's response: both the number of photons and the energy level in x 1 dim blue light (1.8×10^{13} photons/cm^2/sec, 7.8 $\mu w/cm^2$) were

higher than those in red light, either x 1 or x 0.1 dim light (1.2×10^{13} photons/cm^2/sec, 4.0 μw/cm^2 for x 1 dim red light; 1.2×10^{12} photons/cm^2/sec, 0.4 μw/cm^2 for x 0.1 dim red light). Consequently, wavelength seems to be the most important factor of long photoperiod light for the maintenance of mature testes. Differences in light absorption by tissues overlying the brain photoreceptor cannot be discounted, however, as will be discussed later.

The action spectrum for enucleated birds under x 1 dim light showed the same tendency as that for intact birds in x 0.1 dim light: red light - effective, green light - intermediate, and blue light - ineffective. On the other hand, Benoit (1964) reported that the extraretinal photoreceptor of the duck responded to blue light as well as to red light when the light was introduced directly into the brain through a quartz rod. Homma (1969) also showed that the extraretinal photoreceptor in Japanese quail can respond to both blue (455 nm) and orange-yellow (575 nm) light when radioluminescent paint was implanted in some part of the brain (see Section III-A). These reports suggest that the extraretinal photoreceptor can be stimulated by a wide range of wavelengths of light when it is illuminated directly. However, when the extraretinal

photoreceptor is illuminated from outside as in my experiments, only red light is effective. In this case, absorption of light by overlying tissues may be involved. As Benoit and his colleagues reported, long wavelengths can pass through the tissues more easily than shorter wavelengths. Consequently, the different effects of various wavelengths on the extraretinal photoreceptor is probably due to the filtering effect of overlying tissues.

The difference in the action spectrum for the photoperiodic gonadal response, between intact and enucleated birds, can be explained in either of two ways, 1) the eye is one of the photoreceptors, 2) the eye acts only as a "light guide" which focusses the light on the brain photoreceptor. Thus a stimulatory amount of green light penetrated into the brain of intact birds at x 1 dim light because of the light guide effect of the eye, while, in enucleated birds, the light guide is absent so that the results were the same as for intact birds at the lower intensity (x 0.1 dim light). If this is the case, the entire spectrum of visible light could have an effect on gonadal activity under natural conditions, because the intensity of sunlight is presumably high enough even in the blue region to stimulate the brain photoreceptor. Since Schildmacher (1963) showed that a high intensity

(150, 200 lux) of blue light stimulates gonadal growth of intact birds (several Passeriform species), the possibility described above seems quite likely. This question should be checked in blinded birds by using a very high intensity of monochromatic blue light.

In mammals, several reports deal with the effects of wavelength on gonadal activity (in rats: Luce-Clausen and Brown, 1939; Allardyce et al, 1942; Wurtman and Weisel, 1969; in mice: Barbanti-Silva, 1932; in ground squirrels: Johnson and Gann, 1933; in ferrets: Marshall and Bowden, 1934). These investigators can be divided into two groups: some believe that wavelength has a key role in the photo-gonadal reflex, and others believe that all wavelengths of visible light and ultraviolet are effective, while infra-red is not, and that the degree of response depends on the intensity. However, since most of the investigators did not consider the several variables associated with light, it is hard to interpret their results. Recently, Vriend (personal communication) concluded from his experiments on mice that the number of photons is the most important factor in controlling the photo-sexual response and that the apparent effect of wavelength within the visible spectrum can be explained by the total number of photons. If this is the case, the mechanism of photoreception for

the gonadal response might be quite similar between mammals and birds, even though the photoreceptor is different (the eyes for mammals; the extraretinal photoreceptor for birds). However, the integration of the light information received seems to be quite different, because the quantity of light seems to be an important factor of the photoperiod in mammals, while the timing of the light period has the more important role in birds.

Experiment IV and V have confirmed the results of Oishi and Kato (1968), in showing that the pineal may function as a photoreceptor which receives only red light. Homma's negative findings (Homma, 1969) regarding the photoreceptive function of the pineal can be explained on the basis of the different amount of radioluminescent paint he used, or the different phase of the photoperiodic gonadal response he studied. Barfuss and Ellis (1971) showed that red light affected HIOMT activity in the pineal and caused enlarged testes of the house sparrow. Although these authors did not regard the pineal as a photoreceptor, their results concur with mine and also with those reported by Munns (1970) for the canary and Rosner _et al_ (1971) for duck pineal _in vitro_, and suggest that the avian pineal may receive and respond to light of long wavelengths.

IV. PHYSIOLOGICAL AND ULTRASTRUCTURAL STUDY OF THE PINEAL BODY IN RELATION TO THE PHOTOPERIOD AND TO SEVERAL ENDOCRINE GLANDS IN JAPANESE QUAIL

A. EFFECTS OF THE PHOTOPERIOD AND OF PINEALECTOMY ON VARIOUS ENDOCRINE GLANDS

INTRODUCTION

The photosensitivity of the avian pineal was discussed in the previous section. This section concentrates on the endocrine function of the pineal.

In its elaboration of melatonin, which has a chromatophorotropic function in lower vertebrates and an antigonadal function in mammals, the pineal seems to fit the classic definition of an endocrine gland, although the target organ is not clear in birds. The relationship of the avian pineal to other endocrine glands has not been elucidated in spite of extensive study (see, for instance, the contradictory reports reviewed by Kitay and Altschule (1954) and by Ralph (1970)).

In Japanese quail, as in other species of birds, pinealectomy has been reported to be stimulatory, inhibitory, or has had no effect on gonadal activity. Pinealectomy of young quail (operated at 1 week of age) permitted rapid oviducal growth, but had no effect on ovary weight at 4 weeks of age, when birds were reared under 14L/10D.

Pinealectomy was without effect on quail of either sex kept under other photoperiods (24L/0D, 12L/12D or 8L/16D) (Homma *et al*, 1967). On the other hand, Sayler and Wolfson (1967, 1968 a) reported that pinealectomy of juvenile Japanese quail (operated at 7 - 9 days of age) exposed to a stimulatory photoperiod (16L/8D) resulted in delays in ovarian and oviducal development and decreased pituitary weight as measured at 43 - 47 days of age. These effects were transitory and occurred only during the growth period immediately preceding the onset of sexual maturity. Pinealectomy did not have such effects on the gonads of male birds.

Other investigators have reported no effect of pinealectomy on gonadal activity. Pinealectomy did not alter gonadal inhibition in immature quail or gonadal atrophy in mature quail exposed to short photoperiods (Arrington *et al*, 1969). Pinealectomy of 15 - 16 day old quail, subsequently reared under 16L/8D, had no significant effect on testicular weight in males (examined at 46, 49, 61 and 76 days of age), nor was there any significant change in the time of onset of ovulation in females (Renzoni, 1967). Kannankeril (1970) reported that pinealectomy did not alter the hypertrophy of the rudimentary right gonad following sinistral ovariectomy. Thus, the effect of pinealectomy on the gonads seems to depend on the age

and sex of the birds, and on the photoperiod to which the birds are exposed.

Menaker and his colleagues recently have shown for the house sparrow that the pineal is involved in the regulation of circadian locomotor rhythms (Gaston and Menaker, 1968), and circadian body temperature variations (Binkley et al, 1971). However, most other experiments on the avian pineal have focussed on the response to the photoperiod of the pineal - gonadal axis (Ralph, 1970), except for a few studies on the adrenal and thyroid (Nikolaiczuk and Maw, 1942; Kleinpeter and Mixner, 1947). Nikolaiczuk and Maw reported that thyroid and adrenal weights of the chicken were reduced and pituitary and testis weight were increased, by exposure to sunlight (natural photoperiod between May and August or October) in comparison with those without direct sunlight irradiation. On the other hand, Kleinpeter and Mixner reported a stimulatory effect of light on the thyroid in baby chicks.

In mammals, responses of the mouse thyroid to the the photoperiod were reported by Puntriano and Meites (1951), who showed that continuous light induced significant reductions in (a) thyroid wieght, (b) thyroid reaction to thiouracil, and (c) thyroid uptake of radioactive iodine (I^{131}), while continuous darkness produced the opposite effects.

Houssay and Pazo (1968) reported that pinealectomy increased thyroid and adrenal weights in non-hypophysectomized rats and increased thyroid and ovary weights in hypophysectomized rats (photoperiod was not indicated). On the other hand, Bick _et al_ (1969) and Rowe _et al_ (1970) reported that neither the photoperiod (continuous light, diurnal light or continuous darkness) nor pinealectomy had any effect on the pituitary - thyroid axis of rats. Fiske and Lambert (1962) found that after 9 to 10 weeks' exposure to continuous light the adrenals of female rats were significantly smaller than those of controls which had been housed in a naturally lighted room. Males did not show such a change. Nir _et al_ (1971) showed that pinealectomy increased the plasma corticosterone level in female rats kept for 10 days in alternating light or in constant darkness, but this effect disappeared after 30 days. In female hamsters, Reiter _et al_ (1966) reported that exposure of animals to 1L/23D caused a significant reduction in adrenal gland weight, which was prevented by pinealectomy. They further reported that the thyroid glands of dark-exposed (1L/23D), goitrogen-treated animals hypertrophied less than those of similarly treated animals maintained in 16L/8D, while neither of these photoperiods (16L/8D and 1L/23D) nor pinealectomy had any effect on thyroid weight in untreated animals.

Considering the above reports concerning the relationship between the photoperiod and the pineal and endocrine organs in mammals, it seemed of interest to investigate the effects of the photoperiod on the avian pineal body, and the relationship of the pineal in turn to various endocrine glands.

MATERIALS AND METHODS

Experiment I - Effect of the photoperiod and of pinealectomy on various endocrine glands of immature males and females: Immature male and female Japanese quail (2 weeks old) were subjected to pinealectomy or sham operation (sham operated males - Experiment I-A, pinealectomized males - Experiment I-B, sham operated females - Experiment I-C, pinealectomized females - Experiment I-D). The birds were assigned to one of two lighting treatments, to either continuous light (24L/0D) or a short photoperiod (8L/16D). Temperature was maintained at 30.9 ± 0.3 °C. After 3 weeks of the experiment, all birds were killed and body weight, gonad, thyroid, adrenal and pituitary weights were measured.

Experiment II - Effects of the photoperiod on various endocrine glands of adult male and female quail: Adult quail of both sexes (males - Experiment II-A, females - Experiment II-B) were divided at 9.5 weeks of age into two groups, one maintained under a long photoperiod (16L/8D,

lights on from 0600 to 2200) and the other in a short photoperiod (8L/16D, lights on from 0900 to 1700). The temperature was 30.0 ± 0.6 °C. After 3 weeks, body weight and gonad, thyroid, adrenal, pituitary, pineal and spleen weights were measured.

Experiment III - Effect of the photoperiod and of pinealectomy on various endocrine glands of adult male quail: Adult male quail (10.5 weeks old) were subjected to pinealectomy or sham operation (sham operation - Experiment III-A, pinealectomy - Experiment III-B). They were divided into two groups, one assigned to continuous darkness (0L/24D) and the other to 24L/0D. Temperature was 28.3 ± 0.5 °C. After 3 weeks, body weight and thyroid, adrenal and gonadal weights were measured.

RESULTS

Experiment I - Effects of the photoperiod and of pinealectomy on various endocrine glands of immature male and female quail: Immature male quail - The results are shown in Table IX and X. After 3 weeks of lighting treatment, sham operated birds showed considerably higher testis, adrenal and pituitary weights in 24L/0D than in 8L/16D ($p < 0.001$, $p < 0.01$, and $p < 0.01$, respectively, for both absolute and relative (mg organ weight x 100 per gm body weight) values (Tables IX and X, Experiment I-A). Pineal-

ectomy abolished the response of the adrenal and pituitary to the photoperiod (Tables IX and X, Experiment I-B). However, pinealectomy did not alter the reduction of testis weight under 8L/16D. There were no significant differences in body weight or thyroid weight between birds maintained in 24L/0D and 8L/16D. There were no statistically significant differences in organ weights between sham operated and pinealectomized birds within the same lighting treatment.

Immature female quail - The results are shown in Tables XI and XII. Among sham operated birds, body weight, ovary weight, adrenal weight and pituitary weight were significantly higher in 24L/0D than in 8L/16D (Table XI, XII; body weight, $p < 0.01$, ovary weight, $p < 0.02$, for both absolute and relative values; adrenal weight, $p < 0.05$ for absolute and $p < 0.1$ for relative value; pituitary weight, $p < 0.01$ for absolute and $p < 0.05$ for relative value). There were no differences in thyroid weight, in either absolute or relative values, between sham operated birds in 24L/0D and 8L/16D. In pinealectomized birds (Tables XI, XII, Experiment I-D), all of the above differences in organ weights, except for ovary weight, disappeared. The ovary weight of pinealectomized birds, like that of sham operated birds, showed a significant difference between

24L/0D and 8L/16D ($p < 0.05$ for absolute and relative values). There was no statistically significant difference in organ weights between sham operated and pinealectomized birds within the same lighting treatment. Oviduct weight was also measured in 24L/0D birds, but this value was not significantly affected by pinealectomy, although there was a slight difference (1,646 mg in sham operated birds, and 1,193 mg in pinealectomized birds).

Experiment II - Effects of the photoperiod on various endocrine glands of adult male and female quail: The results are shown in Tables XIII and XIV. At 12.5 weeks of age, after 3 weeks of lighting treatment, both absolute and relative gonadal weight was considerably reduced under 8L/16D both in males and females ($p < 0.01$ for males and $p < 0.05$ for females). Absolute pituitary weight was also reduced in both sexes under 8L/16D, although this reduction in females was not statistically significant. There were no significant differences in body weight or other organ weights under 16L/8D as compared to 8L/16D.

Experiment III - Effects of the photoperiod and of pinealectomy on various endocrine glands of adult male quail: The results are shown in Tables XV and XVI. In sham operated birds (Experiment III-A), testis weight (absolute and relative) of birds in 0L/24D was significantly

Table IX

Effects of the photoperiod and of pinealectomy on various endocrine glands of immature male quail (Results of Experiment I-A, I-B in Section IV-A) (absolute organ weights)

Group	Photoperiod	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	Thyroid Weight (mg ± S. E.)	Adrenal Weight (mg ± S. E.)	Pituitary Weight (mg ± S. E.)
Experiment I-A; sham operated birds						
A (12)#	24L/0D	94.8 ± 2.4	999 ± 58	5.87 ± 0.47	5.91 ± 0.27	1.28 ± 0.04
B (11)	8L/16D	90.5 ± 2.5	7.7 ± 0.82****	6.10 ± 0.54	4.59 ± 0.19***	0.98 ± 0.05***
Experiment I-B; pinealectomized birds						
C (8)	24L/0D	90.8 ± 3.2	882 ± 66	5.81 ± 0.63	5.17 ± 0.31	1.21 ± 0.08
D (7)	8L/16D	95.9 ± 2.6	10.5 ± 3.3****	7.25 ± 1.03	5.08 ± 0.25	1.09 ± 0.11

Number of birds

*** $p < 0.01$, **** $p < 0.001$

S. E. standard error

Table X

Effects of the photoperiod and of pinealectomy on various endocrine glands of immature male quail (Results of Experiment I-A, I-B in Section IV-A) (relative organ weights)

Group	Photoperiod	T. Wt/B. W.	Thy Wt/B. W.	Adr Wt/B. W.	Pit Wt/B. W.
Experiment I-A; sham operated birds					
A (12)#	24L/0D	1,054 ± 55	6.19 ± 0.51	6.28 ± 0.34	1.35 ± 0.05
B (11)	8L/16D	8.6 ± 0.82****	6.69 ± 0.49	5.07 ± 0.17***	1.09 ± 0.04***
Experiment I-B; pinealectomized birds					
C (8)	24L/0D	966 ± 53	6.47 ± 0.74	5.71 ± 0.30	1.35 ± 0.10
D (7)	8L/16D	10.8 ± 3.3****	7.56 ± 1.07	5.31 ± 0.26	1.13 ± 0.09

relative organ weight : mg Organ Weight x 100/gm Body Weight

*** $p < 0.01$, **** $p < 0.001$

Number of birds

Table XI

Effects of the photoperiod and of pinealectomy on various endocrine glands of immature female quail (Results of Experiment I-C, I-D in Section IV-A) (absolute organ weights)

Group	Photoperiod	Body Weight (gm ± S. E.)	Ovary Weight (mg ± S. E.)	Thyroid Weight (mg ± S. E.)	Adrenal Weight (mg ± S. E.)	Pituitary Weight (mg ± S. E.)
Experiment I-C; sham operated birds						
E (8)#	24L/0D	106.9 ± 2.0	674 ± 210 (1,646 ± 285)⊥	9.58 ± 2.87	5.62 ± 0.30	1.54 ± 0.11
F (9)	8L/16D	94.2 ± 1.6***	30 ± 0.2**	7.23 ± 0.75	4.47 ± 0.28*	1.03 ± 0.06***
Experiment I-D: pinealectomized birds						
G (8)	24L/0D	92.1 ± 4.7	668 ± 255 (1,193 ± 414)⊥	7.09 ± 0.86	4.66 ± 0.37	1.30 ± 0.09
H (10)	8L/16D	101.2 ± 2.4	33 ± 0.2*	8.36 ± 1.32	4.76 ± 0.28	1.30 ± 0.05

number of birds

⊥ Oviduct Weight

* $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$

Table XII

Effects of the photoperiod and of pinealectomy on various endocrine glands of immature female quail (Results of Experiment I-C, I-D in Seciton IV-A) (relative organ weights)

Group	Photoperiod	O. W./B. W.	Ovi Wt/B. W.	Thy Wt/B. W.	Adr Wt/B. W.	Pit Wt/B. W.
Experiment I-C; sham. operated birds						
E (8)#	24L/0D	628 ± 195	1,536 ± 255	8.77 ± 2.50	5.26 ± 0.25	1.45 ± 0.11
F (9)	8L/16D	32 ± 0.2**	-------	7.61 ± 0.69	4.72 ± 0.24	1.10 ± 0.06*
Experiment I-D: pinealectomized birds						
G (8)	24L/0D	694 ± 265	1,226 ± 403	7.79 ± 1.02	5.05 ± 0.27	1.42 ± 0.08
H (10)	8L/16D	32 ± 0.2*	-------	8.24 ± 1.37	4.63 ± 0.25	1.30 ± 0.06

number of birds

* $p < 0.05$; ** $p < 0.02$

relative organ weight: mg Organ Weight x 100/gm Body Weight

Table XIII

Effects of the photoperiod on various endocrine glands of adult male and female quail (Results of Experiment II-A, II-B in Section IV-A) (absolute organ weights)

	A: 16L/8D	B: 8L/16D
Experiment II-A: male		
Number of Birds	6	8
Body Weight (gm)	112.0 ± 9.1	109.4 ± 2.8
Testis Weight (mg)	1,338 ± 64	458 ± 224***
Thyroid Weight (mg)	3.84 ± 0.22	5.22 ± 0.79
Adrenal Weight (mg)	6.24 ± 0.25	5.87 ± 0.21
Pituitary Weight (mg)	1.92 ± 0.11	1.50 ± 0.10*
Pineal Weight (mg)	0.66 ± 0.05	0.84 ± 0.07
Spleen Weight (mg)	35.4 ± 9.3	51.1 ± 9.4
Experiment II-B: female		
Number of Birds	5	4
Body Weight (gm)	140.6 ± 7.5	130.0 ± 5.1
Ovary Weight (mg)	5,776 ± 815	2,073 ± 815*
Thyroid Weight (mg)	6.88 ± 1.08	5.54 ± 0.69
Adrenal Weight (mg)	7.11 ± 0.26	6.65 ± 0.70
Pituitary Weight (mg)	2.02 ± 0.10	1.68 ± 0.13
Pineal Weight (mg)	0.89 ± 0.06	0.77 ± 0.03
Spleen Weight (mg)	33.4 ± 5.6	62.3 ± 23.2

* $p < 0.05$, *** $p < 0.01$

Table XIV

Effects of the photoperiod on various endocrine glands of adult male and female quail (Results of Experiment II-A, II-B in Section IV-A) (relative organ weights)

	A: 16L/8D	B: 8L/16D
Experiment II-A: male		
Number of Birds	6	8
Tes Wt/B. W.	1,222 ± 92	407 ± 194***
Thy Wt/B. W.	3.54 ± 0.37	4.74 ± 0.65
Adr Wt/B. W.	5.70 ± 0.39	5.40 ± 0.26
Pit Wt/B. W.	1.76 ± 0.16	1.37 ± 0.08
Pin Wt/B. W.	0.60 ± 0.06	0.77 ± 0.06
Spl Wt/B. W.	32.3 ± 9.2	46.9 ± 8.9
Experiment II-B: female		
Number of Birds	5	4
Ova Wt/B. W.	4,051 ± 453	1,534 ± 590*
Thy Wt/B. W.	4.98 ± 0.89	4.28 ± 0.53
Adr Wt/B. W.	5.11 ± 0.34	5.09 ± 0.42
Pit Wt/B. W.	1.47 ± 0.15	1.28 ± 0.10
Pin Wt/B. W.	0.65 ± 0.08	0.59 ± 0.03
Spl Wt/B. W.	24.7 ± 5.35	50.3 ± 21.2

relative organ weight: mg organ weight x 100/gm Body Weight

* $p < 0.05$, *** $p < 0.01$

Tablv XV

Effects of the photoperiod and of pinealectomy on various endocrine glands of adult male quail (Results of Experiment III-A, III-B in Section IV-A)

(absolute organ weights)

Group	Photoperiod	Body Weight (gm ± S. E.)	Testis Weight (mg ± S. E.)	Thyroid Weight (mg ± S. E.)	Adrenal Weight (mg ± S. E.)
Experiment III-A:	sham operated birds				
A (7)#	24L/0D	121.0 ± 5.0	1,371 ± 87	3.34 ± 0.22	6.34 ± 0.49
B (6)	0L/24D	107.5 ± 3.9	353 ± 196***	4.42 ± 0.91	7.36 ± 0.46
Experiment III-B:	pinealectomized birds				
C (7)	24L/0D	119.0 ± 3.6	1,364 ± 132	4.03 ± 0.38	7.92 ± 0.58
D (7)	0L/24D	101.7 ± 2.6***	305 ± 124***	4.64 ± 0.64	6.80 ± 0.42

number of birds

*** $p < 0.01$

Table XVI

Effects of the photoperiod and of pinealectomy on various endocrine glands of adult male quail (Results of Experiment III-A, III-B in Section IV-A) (relative organ weights)

Group	Photoperiod	Tes Wt/B. W.	Thy Wt/B. W.	Adr Wt/B. W.
Experiment III-A: sham operated birds				
A (7)#	24L/0D	1,134 ± 59	2.75 ± 0.11	5.29 ± 0.47
B (6)	0L/24D	336 ± 186***	4.11 ± 0.78	6.82 ± 0.22*
Experiment III-B: pinealectomized birds				
C (7)	24L/0D	1,158 ± 116	3.41 ± 0.35	6.67 ± 0.47
D (7)	0L/24D	286 ± 111***	4.60 ± 0.66	6.67 ± 0.33

number of birds

* $p < 0.05$; *** $p < 0.01$

relative organ weight: mg Organ Weight x 100/gm Body Weight

reduced ($p < 0.01$). Relative adrenal weight was larger under 0L/24D than under 24L/0D, contrary to the results with young birds. However, the absolute value did not show any statistically significant difference. There was no difference between 24L/0D and 0L/24D birds in body weight or in thyroid weight. In pinealectomized birds, (Experiment III-B), body weight and testis weight (both absolute and relative) of animals in 0L/24D were considerably lower than for those in 24L/0D ($p < 0.01$). Pinealectomy abolished the effect of the photoperiod on adrenal weight. Thyroid weight did not differ significantly between 24L/0D and 0L/24D pinealectomized birds.

DISCUSSION

The results of Experiment I revealed possible relationships between the photoperiod and the pineal, pituitary and adrenal in immature quail, while the thyroid gland was unaffected by the photoperiod or by pinealectomy. In adult birds, the effect of the photoperiod on the pituitary and adrenal glands was not very clear (Experiment II and III). The adrenal gland of young birds was shown to be dependent on the pituitary, but this dependence was less marked among adult quail (Section IV-C). This, together with the response of pinealectomized birds to the lighting treatment, suggests that the photoperiod - pineal relation-

ship may be important in the maturation of the pituitary - adrenal axis. Since pinealectomy abolished the response of the pituitary and of the adrenal to the photoperiod, the pineal might be functioning (1) as a photoreceptor, or (2) as a neuroendocrine gland which affects the pituitary - adrenal axis, or (3) both. The examination of these possibilities is left for future investigation.

As was described in the Introduction, there have been few studies on the relationships between the photoperiod, pineal and endocrine glands, except for the gonads, in birds. Inconsistent results on the effects of light on the adrenal (inhibitory by Nikolaiczuk and Maw (1942) in chickens, stimulatory in this paper) and on the thyroid (inhibitory by Nikolaiczuk and Maw (1942), stimulatory by Kleinpeter and Mixner (1947) in baby chicks, and no effect in this paper) could be due to differences in species and age of the birds. However, since temperature differences can reverse the effect of the photoperiod on the thyroid of rats (Soliman <u>et al</u>, 1958) and since light without ultraviolet produced thyroid hyperplasia in the chicken (Turner and Benedict, 1932), the influence of temperature and of the spectral composition of the light must also be considered.

There are four reports which showed no effect of pinealectomy on the adrenal or the thyroid in the chicken

(Mikami, 1950; Shellabarger and Breneman (1950) quoted by Ralph, 1970; Stalsberg, 1965; Zadura et al, 1969). However, since none of these investigators took the photoperiod into consideration, negative results might have been expected according to the results reported in this paper (no statistically significant difference in organ weights between pinealectomized and sham operated birds under the same photoperiod, while pinealectomy abolished the responses of the pituitary and the adrenal to long and short photoperiods). Also of interest in this regard is the finding of Singh and Turner (1967) that melatonin reduced adrenal weight of the 10 week old chicken, while thyroid weight was unaffected.

As was described in the Introduction, reports in mammals suggest that the adrenal gland, but not the thyroid, is sensitive to the photoperiod and to pinealectomy. This is in accordance with the results for Japanese quail reported in this paper. However, continuous light might be acting not only as a long photoperiod but also as a kind of stressor. The pineal might also be involved in the organisms' response to stress. It would be of interest to study the effect of pinealectomy on adrenal changes induced by stress.

The gonadal response to the photoperiod (24L/0D, 8L/16D, or 0L/24D) was not affected by pinealectomy either

in young quail of both sexes or in adult male quail (Experiments I, II, and III). In other experiments I have also found no effect of pinealectomy on the gonads of 21 week old male quail under 12L/12D (Oishi, unpublished data). These confirm the findings of Renzoni (1967), Homma et al (1967), Arrington et al (1969) and Kannankeril (1970). The discrepancy between my results and those of Sayler and Wolfson (1968 a), who showed that pinealectomy delayed ovarian and oviducal development in 35-day old quail under 24L/0D, could be explained as follows: even though the age of the birds was the same in both experiments (35 days), ovarian growth appears to have been slower in birds of my experiment (674 mg) than in quail of their experiment (3,114 mg). Since they showed that the effect of pinealectomy on the ovary was observed at 43 - 47 days when the growth rate of the ovary was slightly retarded by 16L/8D, 35 days in the present experiment might have been just prior to the "critical period" of their experiment. Actually, there was slight difference in oviduct weight between sham operated and pinealectomized birds (Experiment I-C and I-D), suggesting the beginning of a "critical period". It is also possible that age at the time of pinealectomy (2 weeks vs 7 - 9 days in Sayler and Wolfson's work) was responsible for the discrepancy.

B. EFFECT OF LIGHT AND DARKNESS ON PINEAL HIOMT ACTIVITY.

INTRODUCTION

The photoperiodic control of melatonin rhythms is well known in rats (Wurtman et al, 1968). In birds, there are several reports which show the effect of light on activity of the melatonin forming enzyme, hydroxyindole-O-methyl transferase (HIOMT), in the pineal. Higher pineal HIOMT activity in light than in darkness was reported by Axelrod et al (1964) in the female chicken; by Winget et al (1967) in the adult male chicken; by Lauber et al (1968) in male chicks; by Munns (1970) in the canary; by Alexander et al (1970 a, b) in female Japanese quail; by Rosner et al (1971) in male duck pineal in vitro. However, there is one recent report which showed higher HIOMT activity in darkness than in light in 46 - 48 day old Japanese quail, and no difference at 71 days of age (Sayler and Wolfson, 1969). Sex of these birds is not reported.

Axelrod and Lauber (1968) and Alexander et al (1970 a) found evidence for two HIOMT systems in Japanese quail: one requires N-acetyl serotonin as a substrate and O-methylation to produce melatonin; the other requires serotonin as a substrate and O-methylation to produce 5-methoxytryptamine (5-MT). Axelrod and Lauber (1968) fur-

ther reported that the melatonin producing enzyme was heat stable at 48 °C for 4 min. and the other enzyme was heat labile. If this is the case, an enzyme assay conducted at 37 °C or 38 °C, which is even lower than the body temperature of the birds, would not distinguish between the two enzyme systems. Consequently, I have examined the effect of higher incubation temperatures on melatonin production _in vitro_.

MATERIALS AND METHODS

Animal care, source of light, and other details of the experimental design were as described in Section II (General Materials and Methods).

Assay procedure: For assay of HIOMT, the method of Axelrod _et al_ (1961) and Quay (1965) was used with modifications as noted.

Experiment I - The optimum incubation temperature for the melatonin-producing HIOMT assay:

(a) 21 pineals (taken from 11.5 week old male quail) were homogenized in 2.8 ml of pH 7.9 sodium phosphate buffer at 0 °C. After 10 minutes of centrifugation, 0.2 ml of supernatant (1.5 pineal equivalents) was transfered into each of 12 test tubes, and 0.05 ml of N-acetyl serotonin*

---

* Fisher Scientific Co., Fair Lawn, New Jersey

(0.5 mg/ml) was added to 6 of the tubes. The same volume of distilled water was added to the 6 control tubes. Ten λ of S-adenosyl-L methionine (methyl-^{14}C)* (specific activity 0.5 mCi/mM) was added to each tube. Immediately thereafter, pairs of test tubes were incubated for 90 minutes as follows: (1) at room temperature (21 °C), (2) in a water bath at 36 °C, (3) 40 °C, (4) 41.5 °C, (5) 45 °C, (6) 51.5 °C. Incubation was terminated with the addition of 0.5 ml of 0.1 N·NaOH saturated with NaCl. The ^{14}C-melatonin formed was extracted into 6 ml of chloroform. The aqueous layer was removed by aspiration and the chloroform was washed twice with distilled water. A 4 ml aliquot of extract was transferred to a glass vial and evaporated. After evaporation, 1 ml of ethanol and 10 ml of fluor (PPO** 3 g, POPOP*** 100 g, toluene 1,000 ml) were added. Radioactivity was counted for 10 minutes with a Nuclear Chicago scintillation counter (Mark 1)****.

(b) Since the first experiment revealed the optimum temperature for pineal HIOMT activity to be between 45 °C

---

* Amersham/Searle Co., 2000 Nuclear Drive, Des Plaines, Illinois

** Aldrich Chemical Co., Inc., Milwaukee, Wisconsin

*** Aldrich Chemical Co., Inc., Milwaukee, Wisconsin

**** Nuclear-Chicago Co., Des Plaines, Illinois

and 50 °C, a second experiment was set to determine the precise optimum temperature. Twelve pineals from 12 week old male quail were pooled and homogenized in 1.6 ml phosphate buffer, and 0.2 ml of pineal extract (1.5 pineal equivalents) was put into each tube. Incubation temperature was 43 °C, 47 °C or 50 °C. The procedure was otherwise the same as in the first experiment.

Experiment II - Effects of light and darkness on pineal HIOMT activity:

(a) 35-day old male and female quail reared under 24L/0D were divided into 4 groups. During the experimental period group A (males) and group C (females) were kept in continuous light (24L/0D) and group B (males) and group D (females) were kept in continuous darkness (0L/24D).

After 11 days of the experiment, all birds were killed by decapitation between 0900 and 1030. The birds of groups B and D were killed in darkness. Body weights, gonad and pineal weights were recorded. Pineal bodies were dissected out quickly, put in a small plastic vial and immediately frozen on dry ice. HIOMT activity of individual pineals was assayed according to the method described above, with incubation temperature set at 47 °C.

(b) Quail reared under 24L/0D were divided at 30 days of age into four groups; group A (males) and group C (females) were kept in 24L/0D and group B (males) and

group D (females) were kept in 0L/24D. Temperature was 29.2 ± 0.6 °C.

After 20 days of the experiment, all the birds were killed between 0800 and 1200. The birds of groups B and D were killed in darkness. Pineal bodies were dissected out, weighed and frozen for later assay. Body weight and gonadal weight were also recorded.

(c) Male quail reared under 24L/0D to 75 days of age were divided into two groups: group A birds were kept in 24L/0D and group B in 0L/24D. Temperature was 27.0 ± 0.4 °C.

After 21 days of the experiment, all birds were killed between 0800 and 1030. Birds of group B were killed in darkness. Pineal weight, body weight and testis weight were recorded.

(d) Male quail, 21 weeks old and reared under 24L/0D, were divided into two groups: group A birds were maintained in a 12L/12D photoperiod with the light turned on at 0600 and off at 1800; group B birds were kept in 12D/12L, with the light on at night between 1800 and 0600.

After 15 days of the experiment, half of the birds from each lighting treatment were killed at 2400 and half at 1200. Group A birds at 2400 and group B birds at 1200 were killed in darkness. Pineal bodies from 5 birds in each lighting treatment were pooled and homogenized

together. The other procedures were the same as above.

RESULTS

Experiment I - The optimum incubation temperature for the melatonin-producing HIOMT assay: The first experiment revealed that the optimum temperature for incubation of the pineal in vitro was between 45 °C and 50 °C (solid line in Fig. 17). The second experiment determined that the optimum temperature was 47 °C (broken line in Fig. 17). The quails' body temperature, measured in the cloaca, was 38.2 °C and deep body temperature was 42.5 °C. Thus this experiment revealed that the temperature optimum for HIOMT activity is higher than the deep body temperature.

Experiment II - Effects of light and darkness on pineal HIOMT activity:

(a) The results are shown in Table XVII and Fig. 18. At 46 days of age, HIOMT activity of both males and females did not show a statistical difference between 24L/0D and 0L/24D after 11 days of light treatment. Testis weight in 0L/24D was reduced considerably ($p < 0.001$). The ovary was not significantly reduced in weight after 11 days of lighting treatment, although some of the birds' ovaries had started to regress.

(b) After 20 days of lighting treatment, both male

and female quail (50 day old) showed high HIOMT activity in 24L/0D and low activity in 0L/24D (cpm/mg pineal in male and cpm/pineal in female showed statistically significant differences; $p < 0.05$; Table XVII, Fig. 19). Testis weight and ovary weight were reduced considerably in 0L/24D ($p < 0.001$ and $p < 0.01$, respectively). Pineal weights of male quail under 24L/0D were lower than under 0L/24D ($p < 0.05$). There was no difference in pineal weights of female quail between 24L/0D and 0L/24D.

(c) 96 day old male quail, after 21 days of lighting treatment, showed significantly higher HIOMT activity in 24L/0D than in 0L/24D ($p < 0.02$ for both cpm/pineal and cpm/mg pineal; Table XVII, Fig. 20). There was no significant difference in pineal weight. Testis weight in 0L/24D showed a significant reduction ($p < 0.001$).

(d) In 12L/12D, HIOMT activity was high at 1200 (during the light period) and low at 2400 (during the dark period; Table XVII, Fig. 21). When the light-dark cycle was reversed to 12D/12L, HIOMT activity was also reversed (high activity at 2400 during the light period, and low activity at 1200 during the dark period). There were no differences in body weight, pineal weight, or testis weight.

Table XVII

Effect of light and darkness on pineal HIOMT activity

(Results of Experiment II in Section IV-B)

Group	Sex	Photoperiod	Body Weight (gm ± S. E.)	Gonadal Weight (mg ± S. E.)	Pineal Weight (mg ± S. E.)	HIOMT activity/hour cpm/pineal	HIOMT activity/hour cpm/mg pineal
Experiment II (a): 11 day light treatment; 46 days of age							
A (9)#	M	24L/0D	104.2 ± 3.2	1,292 ± 48	----------	6,014 ± 1,016	----------
B (9)	M	0L/24D	92.8 ± 1.7	366 ± 70****	----------	6,092 ± 971	----------
C (9)	F	24L/0D	123.4 ± 3.0	4,333 ± 573	----------	7,108 ± 1,206	----------
D (7)	F	0L/24D	117.3 ± 2.5	4,311 ± 1,260	----------	9,767 ± 779	----------
Experiment II (b): 20 days light treatment; 50 days of age							
A (9)	M	24L/0D	109.9 ± 4.1	1,550 ± 105	0.643 ± 0.026	5,720 ± 963	9,233 ± 1,695
B (5)	M	0L/24D	104.6 ± 1.9	38 ± 12****	0.748 ± 0.025*	3,787 ± 635	5,124 ± 918*
C (7)	F	24L/0D	123.6 ± 4.7	5,200 ± 840	0.774 ± 0.046	4,791 ± 457	6,177 ± 398
D (7)	F	0L/24D	112.1 ± 5.1	537 ± 487***	0.727 ± 0.059	2,789 ± 677*	4,080 ± 997

Table XVII - continued

Group	Sex	Photoperiod	Body Weight (gm ± S. E.)	Gonadal Weight (mg ± S. E.)	Pineal Weight (mg ± S. E.)	HIOMT activity/hour cpm/pineal	HIOMT activity/hour cpm/mg pineal
Experiment II (c): 21 days light treatment; 96 days of age							
A (7)#	M	24L/0D	105.4 ± 4.8	1,143 ± 71	0.78 ± 0.04	5,731 ± 281	7,433 ± 420
B (7)	M	0L/24D	99.7 ± 2.1	77 ± 51****	0.81 ± 0.05	3,940 ± 417**	4,968 ± 558**
Experiment II (d): 15 days of light treatment; 162 days of age							
A⊥ (5)	M	12L/12D	110.6 ± 4.5	464 ± 179	0.572 ± 0.020	5,248	9,175
B° (5)	M	12L/12D	110.8 ± 4.5	270 ± 95	0.610 ± 0.054	4,214	6,908
C° (5)	M	12D/12L	92.8 ± 5.0	482 ± 127	0.650 ± 0.022	4,690	7,292
D⊥ (5)	M	12D/12L	101.6 ± 5.0	290 ± 39	0.646 ± 0.034	6,988	10,163

S. E. standard error

number of birds

⊥ birds were killed in light

° birds were killed in darkness

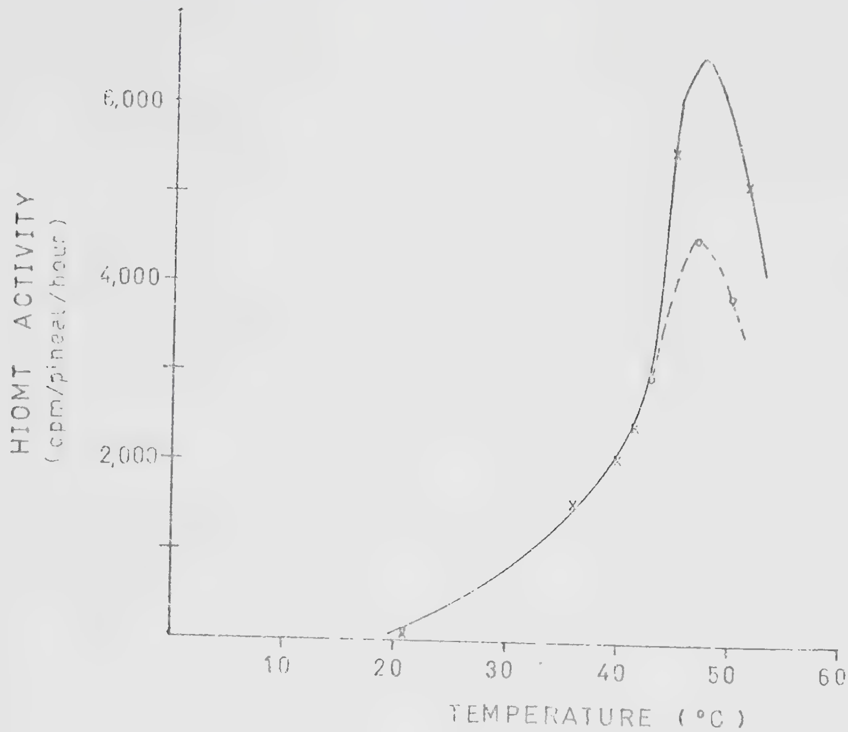
* $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$; **** $p < 0.001$

Figure 17. Incubation temperature optimum for HIOMT assay *in vitro*. (Result of Experiment I in Section IV-B)

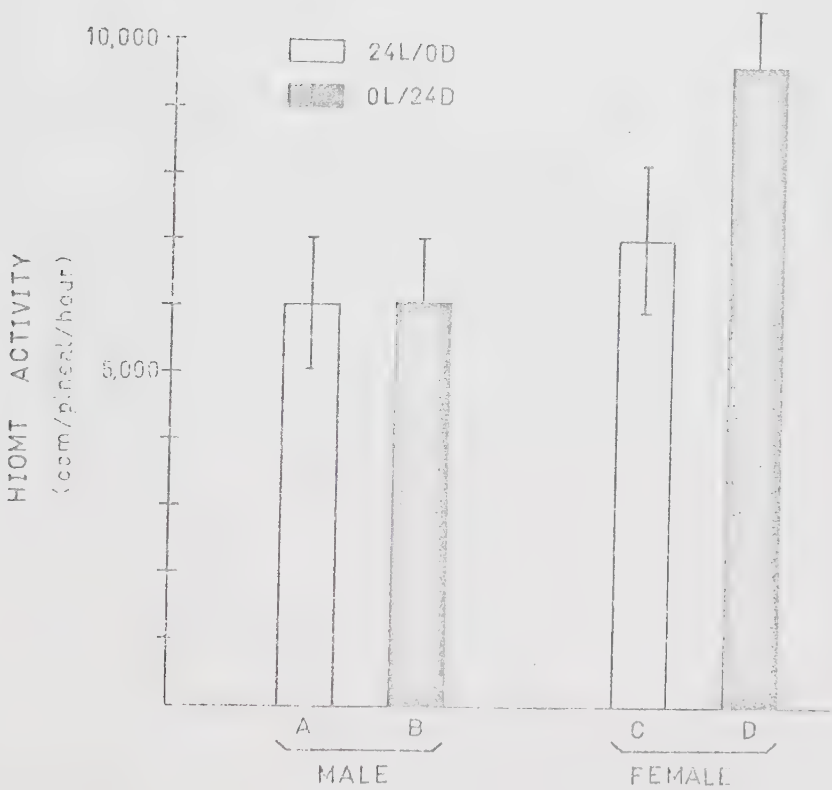
Solid line: Result of Experiment I-(a)

Broken line: Result of Experiment I-(b)

Figure 18. Pineal HIOMT activity after 11 days in 24L/0D or 0L/24D (46 day old male and female quail) (Results of Experiment II (a) in Section IV-B) The vertical bar represents standard error.



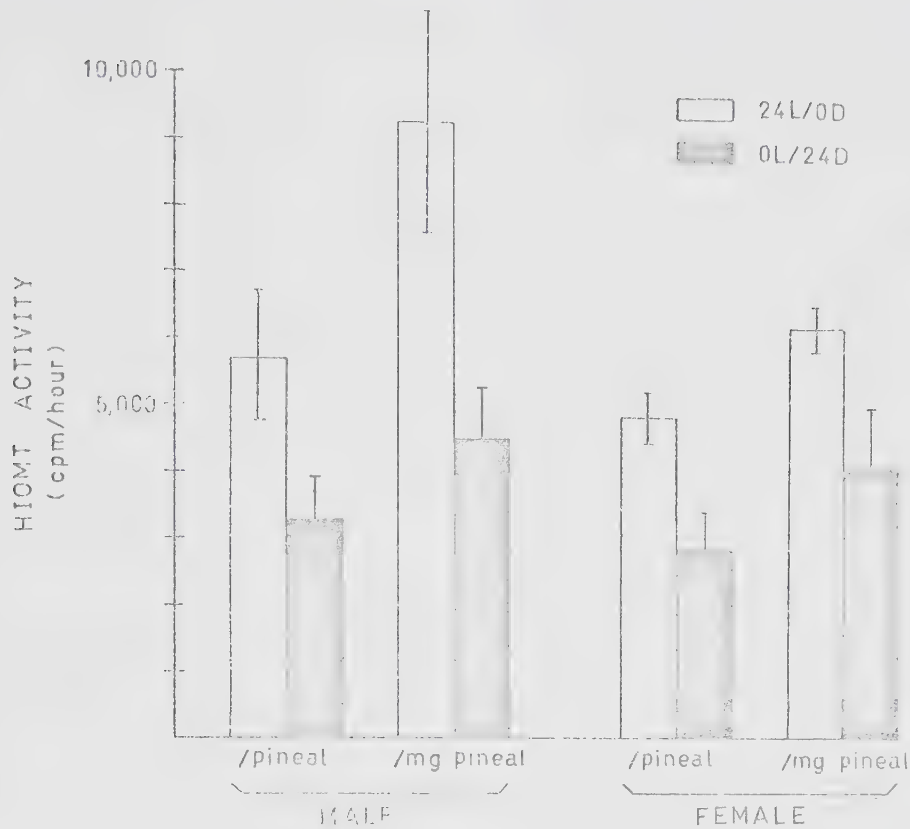
6,000
4,000
2,000
HIOMT ACTIVITY
(cpm/pineal/hour)
10
20
30
40
50
60
TEMPERATURE (°C)



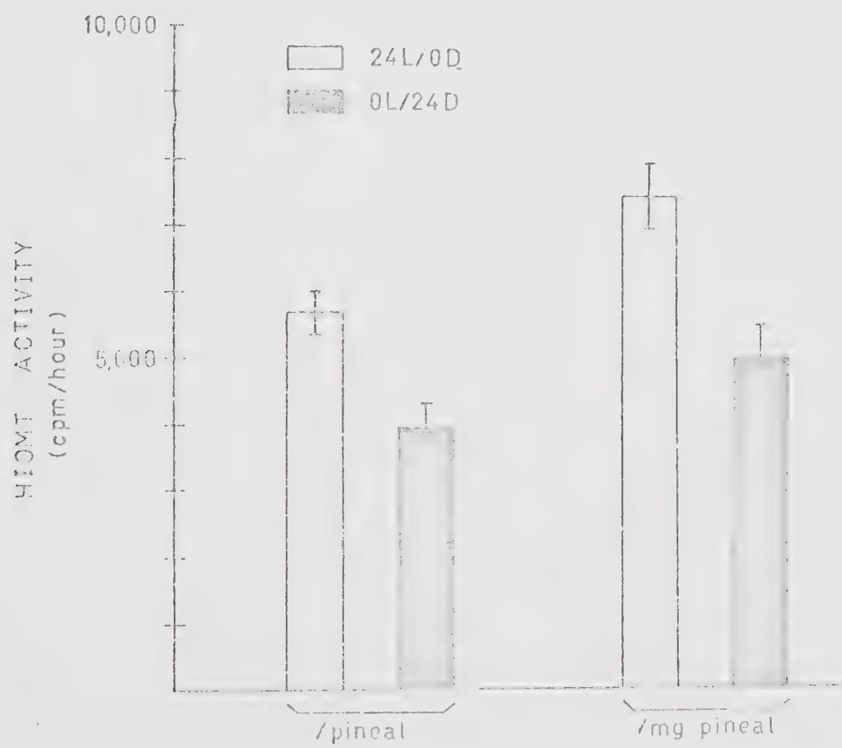
10,000
5,000
24L/0D
0L/24D
HIOMT ACTIVITY
(cpm/pineal/hour)
A
B
C
D
MALE
FEMALE

Figure 19. Pineal HIOMT activity after 20 days in 24L/0D or 0L/24D (50 day old male and female quail). (Results of Experiment II-(b) in Section IV-B) The vertical bar represents standard error.

Figure 20. Pineal HIOMT activity after 21 days in 24L/0D or 0L/24D (96 day old male quail) (Results of Experiment II-(c) in Section IV-B) The vertical bar represents standard error.

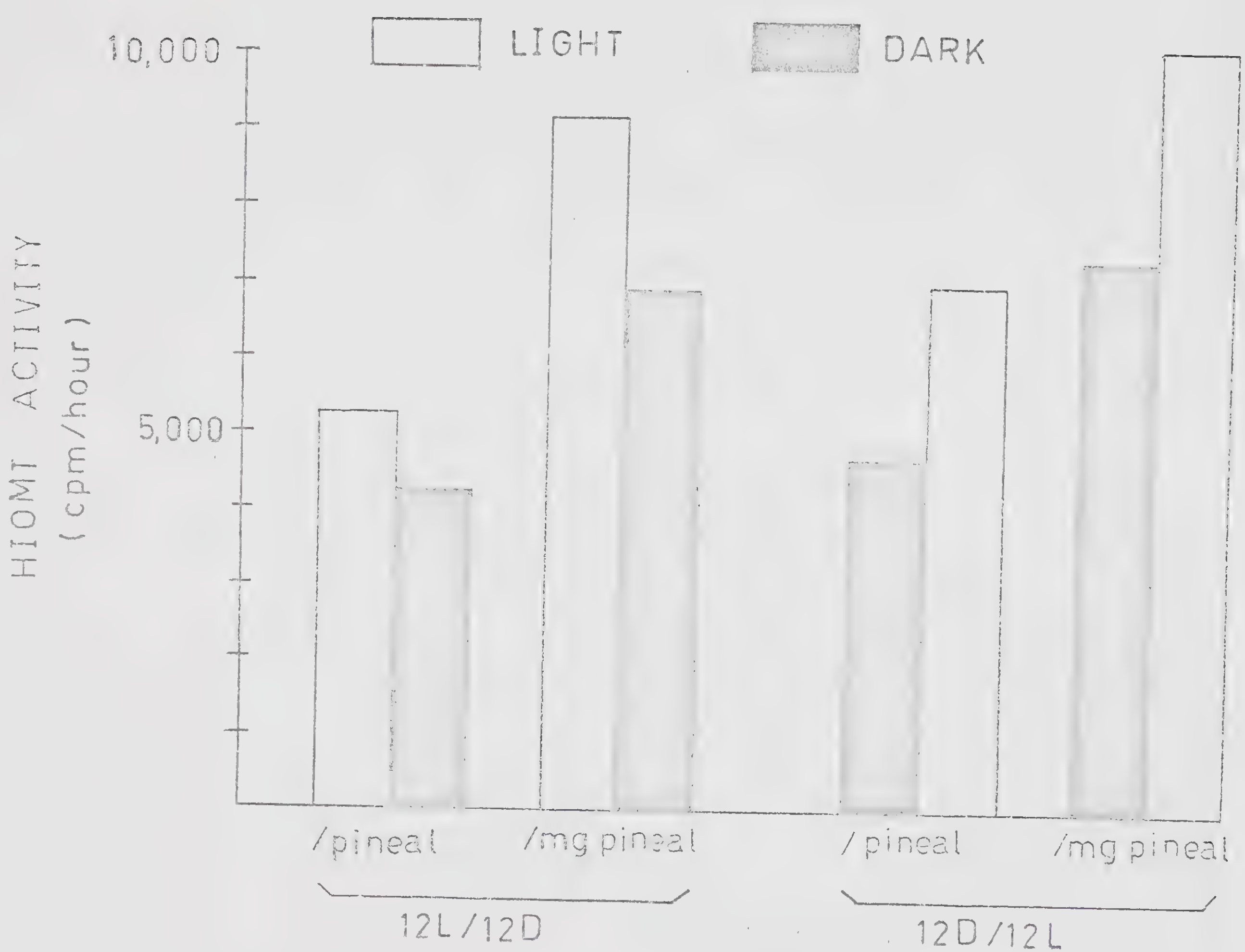


10,000
5,000
HIOMT ACTIVITY
(cpm/hour)
24L/0D
0L/24D
/pineal
/mg pineal
/pineal
/mg pineal
MALE
FEMALE



10,000
5,000
HIOMT ACTIVITY
(cpm/hour)
24L/0D
0L/24D
/pineal
/mg pineal

Figure 21. Pineal HIOMT activity after 15 days in 12L/12D or 12D/12L (162 day old male quail). (Results of Experiment II (d) in Section IV-B)



LIGHT
DARK
HIOMT ACTIVITY
(cpm/hour)
10,000
5,000
/pineal
/mg pineal
/pineal
/mg pineal
12L/12D
12D/12L

DISCUSSION

Quail pineals were assayed for the melatonin-forming enzyme hydroxyindole-O-methyltransferase (HIOMT), with N-acetyl serotonin as substrate. The optimum incubation temperature was determined to be 47 °C. Daytime deep body temperature of the quail was 42.5 °C. Thus the optimum temperature for the enzyme is higher than the body temperature. The enzyme activity at 47 °C was more than three times higher than that at 38 °C. This confirms the report by Axelrod and Lauber (1968) that HIOMT requiring N-acetyl serotonin as substrate was stable after heating at 48 °C for 4 min. The HIOMT activity curve at different incubation temperatures (Fig. 17) showed a single peak, suggesting that only one enzyme is involved in the production of melatonin from N-acetyl serotonin, although there may be another HIOMT requiring serotonin and having a lower temperature optimum (Axelrod and Lauber, 1968; Alexander _et al_ 1970 a).

The finding of high HIOMT activity in continuous light and in the light phase of 12L/12D and 12D/12L lighting schedules, and low activity in continuous darkness, and in the dark phase of 12L/12D and 12D/12L, confirmed the findings by Axelrod _et al_ (1964), Winget _et al_ (1967), Lauber _et al_ (1968), Alexander _et al_ (1970 a, b) and

Rosner *et al* (1971) in various avian species. Sayler and Wolfson (1969) reported high HIOMT activity in the dark phase and low activity in the light phase of 16L/8D in 46 - 48 day old quail but no photoperiod-related difference in HIOMT activity at 71 days of age. The discrepancy between their results and mine might be due to a difference in history of light treatment under which the birds were reared, or to the length of time of the experiment, because it does not seem to be due to a difference in sex or age of the birds. This should be examined in future investigations.

A comparison of the results of Experiments II-(a) and II-(b) is interesting. At least in young quail (46 - 50 days old), 11 days of light treatment did not elicit a difference in enzyme activity but 20 days of treatment did produce an effect. These results suggest that it requires some time for the amount of the enzyme to change after transfer of the animals to a certain light treatment. In the chicken (Axelrod *et al*, 1964) and in 66 day old Japanese quail (Alexander *et al*, 1970 b), 5 days of light treatment was enough to induce a change in HIOMT activity. The reason for this might be the difference in light schedule before the experiment or the different age of the animals at the time of the experiment.

Not only HIOMT, but melatonin itself is reported

to have a rhythm which is regulated by the light and dark cycle (Ralph et al, 1967; Lynch, 1971). However, the maximum amount of melatonin was observed in the dark period and the minimum in the light period, thus the phase of the melatonin rhythm is just opposite to HIOMT activity. However, since a number of events may be involved in these phenomena, the phase of the rhythm need not be expected to coincide. For example, 1) the amount of melatonin produced in vivo is affected not only by the amount of enzyme but also by the concentrations of both substrate and methyl doner, 2) a high amount of pineal melatonin might reflect accumulation in darkness because of inhibition of the release of this substance, 3) Ralph and his colleagues measured the amount of melatonin by melanophore-contracting potency of the crude pineal extract. There may be other substances in the pineal which have the same effect on melanophore contraction as melatonin.

Barfuss and Ellis (1971) recently reported that continuous red incandescent light inhibited HIOMT activity, while light emitted by a fluorescent tube was stimulatory. They explained their results by pointing out that the fluorescent tube provides little energy in the red end of the spectrum. However, incandescent light, which delivers a large fraction of its light in the red, was stimulatory

to HIOMT, according to results reported in this paper and by Rosner et al (1971) and presumably some of the other investigators who showed stimulatory effects of light on enzyme activity may have utilized incandescent light. The solution of the discrepancy concerning action spectrum effects on HIOMT activity in the pineal remains for future investigations.

HIOMT in the house sparrow was found to be inversely related to the annual cycle of gonadal activity, (Barfuss and Ellis, 1971). Alexander et al (1970 a) reported that pineal HIOMT activity in quail increased and subsequently plateaued while the ovary grew from 20 to 90 mg, then HIOMT decreased at the beginning of rapid yolk deposition. These reports suggest a relationship between melatonin and the gonads. However, experiments seeking to show an effect of administered melatonin on gonadal activity yielded inconsistent results. Homma et al (1967) implanted pellets containing melatonin (1,110 or 100 μg) under the skin of 1 week old Japanese quail and observed an inhibitory effect on both male and female gonads after 3 weeks of treatment. Singh and Turner (1967) injected melatonin (50 μg/100 gm. B. W. and 100 μg/100 gm. B. W.) into male and female chickens from 8 to 10 weeks of age, and observed an inhibitory effect on the gonads. On the other hand, Renzoni

(1968) observed no effect of injected melatonin (30, 100 or 500 μg) on the gonads of Japanese quail (males, 15 - 45 days of age; and females, 15 - 100 days of age). Sayler and Wolfson (1969) injected adult Japanese quail with large doses of melatonin (1, 10 or 20 mg) subcutaneously, and found no effect. Oishi (unpublished data) injected 0.1, 1, 10 and 100 μg of melatonin intravenously into adult male Japanese quail maintained under various photoperiods, and found no effect on the gonads. Consequently, although the relationship between light and gonadal activity is well established, the relationship between melatonin and gonadal activity remains to be elucidated.

As was described in Section IV-A, the pineal is involved in photoperiodic pituitary and adrenal responses. Singh and Turner (1967) reported that melatonin had an inhibitory effect on adrenal weights of male and female chickens and a stimulatory effect on the pituitary weight of female chickens. Consequently, melatonin might be an active agent of the pineal which regulates photoperiodically induced pituitary and adrenal responses.

C: ENDOCRINE EFFECTS OF HYPOPHYSECTOMY IN JAPANESE QUAIL

INTRODUCTION

The tropic and feedback relationship between the mammalian hypothalamus-hypophyseal system and several endocrine glands (gonads, thyroid, adrenal cortex) has been well established (Gorbman and Bern, 1966). However, in birds, hypophysectomy has not always led to the "expected" effects on adrenal and thyroid (Miller and Riddle, 1942; Nalbandov and Card, 1943; Newcomer, 1959; Miller, 1961; Ma and Nalbandov, 1963; Nagra *et al*, 1963; Boissin *et al*, 1966; Bradley and Holmes, 1971).

As was shown in Section IV-A, considerably higher pituitary and adrenal weight in continuous light than in a short photoperiod was observed in young Japanese quail, and this difference induced by the photoperiod was abolished by pinealectomy. These reuslts suggested some relationship between the environmental photoperiod and the pituitary and between the photoperiod and the adrenal, but whether there is a direct relationship between the pituitary and the adrenal or thyroid is not clear. To clarify this, experiments were designed to determine the effects of hypophysectomy on various endocrine glands in Japanese quail.

Hypophysectomy has been reported to alter pineal ultrastructure (Lupulescu, 1968; Satodate *et al*, 1970;

Karasek, 1971), and, on the other hand, hypophysectomy had no effect on pineal HIOMT activity in mammals (Wurtman et al, 1964). In this study, I have examined the effects of hypophysectomy on the avian pineal, especially with respect to HIOMT activity.

MATERIALS AND METHODS

Technique of hypophysectomy: Although the trans-buccal method is commonly used for hypophysectomy of birds (Hill and Parkes, 1934; Opel, 1969), Rothchild's oral method (1948) was used because it is a somewhat simpler operation and yielded a higher survival rate (80% survival in adult quail without replacement therapy 2 weeks after the operation). In young quail, especially males, it was difficult to achieve a high survival rate.

Experiment I - Effect of hypophysectomy in immature quail: Male and female quail, reared to 4.5 weeks of age under continuous light (24L/0D), were submitted to either hypophysectomy or sham operation. After surgery, all birds were kept for 13 days in environment chambers under 24L/0D. Animal care and lighting conditions were as described in Section II. Temperature was 30.8 ± 0.2 °C. At the end of the experiment, birds were decapitated and autopsied. The pineal body was dissected out quickly, weighed and put into a plastic vial on dry ice, to be stored

frozen for later measurement of HIOMT activity according to the assay method described in Section IV-B. Body weight, and adrenal, thyroid, and gonad weights were also measured. Completeness of the hypophysectomy was checked anatomically at the time of autopsy.

Experiments II, III, IV, V, VI - Effects of hypophysectomy in adult quail: Male quail were reared under 24L/0D to 8 weeks (Experiment II), 10.5 weeks (Experiment III), 11 weeks (Experiment IV), and 15.5 weeks of age (Experiment VI). Female quail were reared under 24L/0D to 11.5 weeks of age (Experiment V). After hypophysectomy, birds were kept under 24L/0D in environmental chambers. Other procedures were the same as in Experiment I. Temperature was about 28 °C. After 14 days (Experiment II, IV), 10 days (Experiment II), 15 days (Experiment V) and 9 - 10 days (Experiment VI), birds were killed by decapitation and organ weights were measured. Pineal bodies of birds in Experiments IV and V were stored frozen for later HIOMT assay.

RESULTS

In all of these experiments, hypophysectomized birds showed conspicuous molting.

Experiment I - Effect of hypophysectomy in immature quail: All the hypophysectomized male quail died

before the end of the experiment. Results from 5 females surviving out of 11 are shown in Tables XVIII and XIX and Figure 22. Hypophysectomized birds showed considerable reduction of ovary weight in comparison with sham operated birds ($p < 0.001$). Adrenal weight was also reduced considerably ($p < 0.05$), while pineal weight and thyroid weight were not affected by hypophysectomy. Body weight was lower in hypophysectomized birds, but this difference is due to the reduction of ovary weight: when ovary weight was subtracted from body weight, the latter did not differ from normal. There was no difference in HIOMT activity per pineal or per mg pineal between sham operated and hypophysectomized birds (Table XIX, Figure 22).

Experiments II, III, IV, V, VI - Effect of hypophysectomy in adult quail: In Experiment II, hypophysectomized male quail (10 weeks old, 14 days after operation) showed considerably higher body weight ($p < 0.05$) and lower testis and adrenal weights ($p < 0.001$, $p < 0.05$, respectively) than controls (Table XVIII). There was no significant difference in thyroid weight. In Experiment III, hypophysectomized male quail (12 weeks old, 10 days after operation) showed considerable reduction of testis weight ($p < 0.001$). There was no difference in body weight, pineal weight, thyroid weight, or adrenal weight (Table

XVIII). In Experiment VI, hypophysectomized male quail (17 weeks old, 9 - 10 days after operation) showed considerable reduction of testis weight ($p < 0.001$, Table XVIII). There was no difference in body weight, pineal weight, or thyroid weight. In Experiments IV and V, HIOMT activity per pineal and per mg pineal did not show any significant difference between sham operated and hypophysectomized adult male or female quail (Table XIX, Figures 23 and 24). Pineal weight was not affected by hypophysectomy in either males or females. Gonad weight was significantly reduced ($p < 0.001$) after hypophysectomy in both males and females (Table XVIII). Body weight was reduced after hypophysectomy, but when body weights minus testis weights were compared there was no difference between hypophysectomized and sham operated birds.

DISCUSSION

Neither pineal weight nor pineal HIOMT activity showed any change after hypophysectomy either in young (female) or in adult (male and female) quail. This confirms the results of Wurtman et al (1964) on rats. The gonads showed considerable atrophy after hypophysectomy, suggesting their complete dependency on the pituitary. This response of the gonads to hypophysectomy is in agreement with the reports on other avian species (Nalbandov

Figure 22. Pineal HIOMT activity of hypophysectomized birds 13 days after operation (6.5 week old female quail) (Results of Experiment I in Section IV-C)

The vertical bar represents standard error.

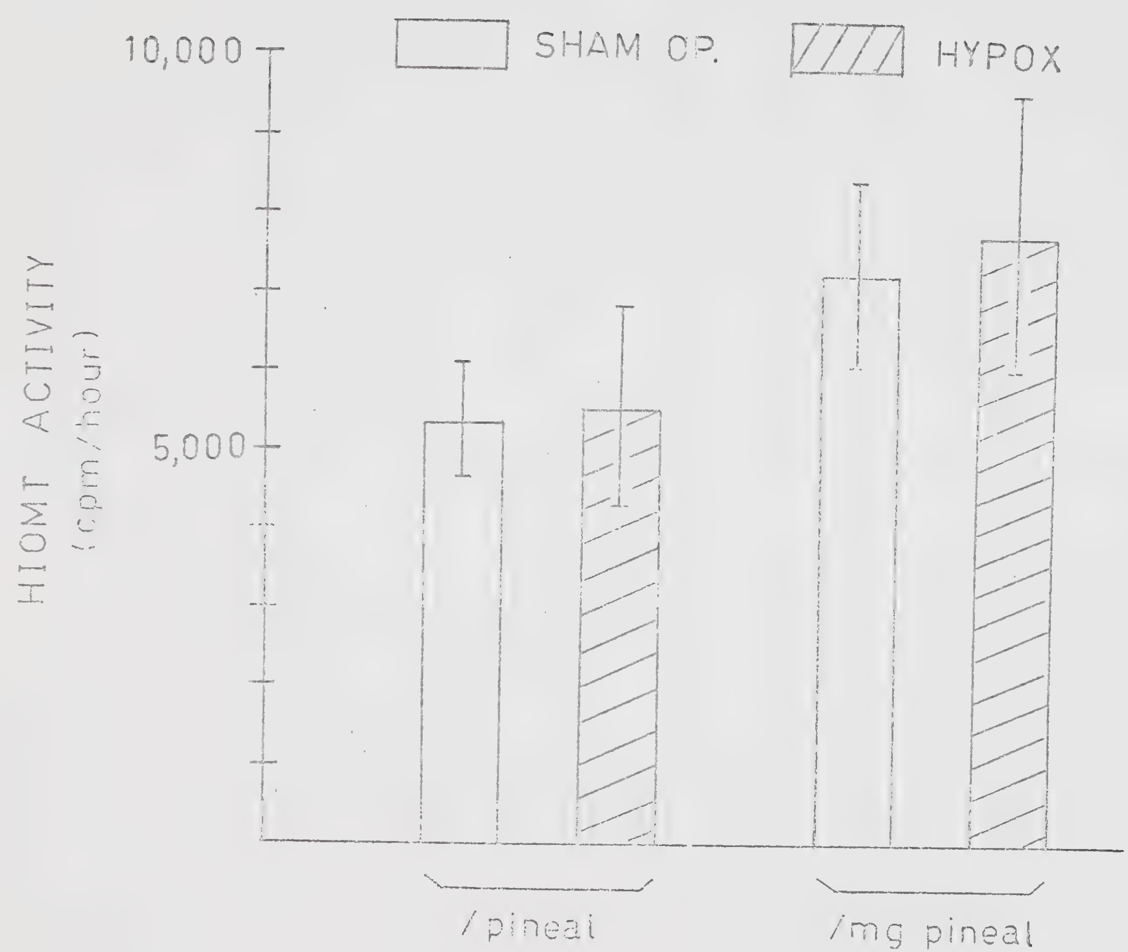
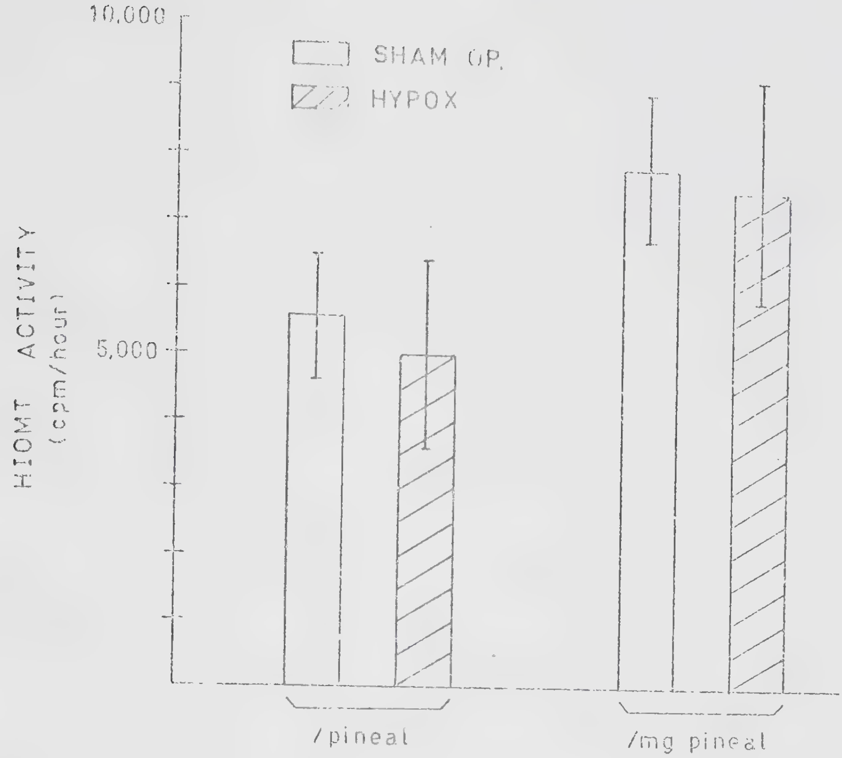

SHAM OP.
HYPOX
HIOMT ACTIVITY
(cpm/hour)
10,000
5,000
/pineal
/mg pineal

Figure 23. Pineal HIOMT activity of hypophysectomized birds 2 weeks after operation (13 week old male quail) (Results of Experiment IV in Section IV-C)
The vertical bar represents standard error.

Figure 24. Pineal HIOMT activity of hypophysectomized birds 2 weeks after operation (13.5 week old female quail) (Results of Experiment V in Section IV-C)
The vertical bar represents standard error.


10,000
5,000
SHAM OP.
HYPOX
HIOMT ACTIVITY
(cpm/hour)
/pineal
/mg pineal

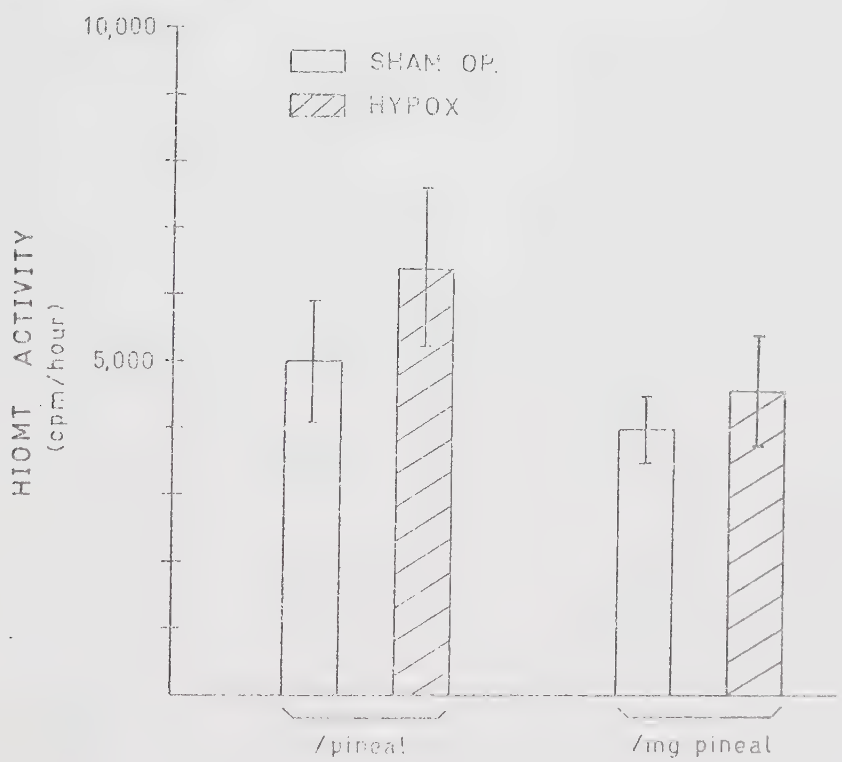

10,000
5,000
SHAM OP.
HYPOX
HIOMT ACTIVITY
(cpm/hour)
/pineal
/mg pineal

Table XVIII

Effect of hypophysectomy on various endocrine glands of quail under continuous light

(Results of Experiments I, II, III, IV, V, and VI in Section IV-C)

Group	Number of Birds	Body Weight (gm ± S. E.)	Pineal Weight (mg ± S. E.)	Gonadal Weight (mg ± S. E.)	Thyroid Weight (mg ± S. E.)	Adrenal Weight (mg ± S. E.)
Experiment I: 6.5 weeks old female; 13 days after operation						
Sham op.	6	120.3 ± 3.1	0.762 ± 0.054	6,608 ± 706	6.60 ± 0.31	7.89 ± 1.05
Hypx.	5	98.8 ± 7.3	0.718 ± 0.067	74.8 ± 32.3^{****}	6.99 ± 1.85	5.41 ± 0.30^{*}
Experiment II: 10 weeks old male; 14 days after operation						
Sham op.	10	109.6 ± 2.8	------------	1,266 ± 71	3.64 ± 0.28	7.17 ± 0.51
Hypx.	3	119.3 ± 1.7	------------	25.0 ± 2.9^{****}	4.19 ± 0.92	5.15 ± 0.33^{*}
Experiment III: 12 weeks old male; 10 days after operation						
Sham op.	7	107.7 ± 6.0	1.260 ± 0.160[1]	1,223 ± 109	4.64 ± 0.27	6.57 ± 0.48
Hypx.	6	107.1 ± 4.2	1.027 ± 0.061[1]	48.6 ± 3.9^{****}	4.28 ± 0.31	5.72 ± 0.45

Table XVIII - continued

Group	Number of Birds	Body Weight (gm ± S. E.)	Pineal Weight (mg ± S. E.)	Gonadal Weight (mg ± S. E.)	Thyroid Weight (mg ± S. E.)	Adrenal Weight (mg ± S. E.)
Experiment IV: 13 week old male; 14 days after operation						
Sham op.	7	108.1 ± 2.9	0.711 ± 0.040	1,370 ± 89	------------	------------
Hypx.	7	98.7 ± 2.4	0.639 ± 0.041	103 ± 77****	------------	------------
Experiment V: 13.5 week old female; 15 days after operation						
Sham op.	9	136.8 ± 4.1	1.254 ± 0.123[1]	4,188 ± 279	------------	------------
Hypx.	7	128.1 ± 3.8	1.370 ± 0.061[1]	86 ± 11****	------------	------------
Experiment VI: 17 week old male; 9 - 10 days after operation						
Sham op.	12	101.1 ± 3.3	1.023 ± 0.060[1]	1,127 ± 91	3.10 ± 0.25	------------
Hypx.	16	99.6 ± 1.4	1.165 ± 0.040[1]	35.2 ± 2.8****	3.13 ± 0.30	------------

[1] pineal weight with choroid plexus

* $p < 0.05$, **** $p < 0.001$

Table XIX

Effect of hypophysectomy on pineal HIOMT activity

(Results of Experiments I, IV, and V in Section IV-C)

Group	HIOMT activity/hour	
	cpm/pineal	cpm/mg pineal
Experiment I: 6.5 week old female; 13 days after operation		
Sham operation	5,382 ± 728	7,217 ± 1,195
Hypophysectomy	5,578 ± 1,295	7,760 ± 1,754
Experiment IV: 13 week old male; 14 days after operation		
Sham operation	5,625 ± 897	7,889 ± 1,129
Hypophysectomy	5,025 ± 1,371	7,527 ± 1,682
Experiment V: 13.5 week old female; 15 days after operation		
Sham operation	5,088 ± 867	4,005 ± 559
Hypophysectomy	6,502 ± 1,177	4,641 ± 775

and Card, 1943; Baum and Meyer, 1956; Nagra _et al_, 1963, in the chicken; Baylé _et al_, 1970, in Japanese quail; Bradley and Holmes, 1971, in the duck).

In Japanese quail, my work revealed no change in thyroid weight after hypophysectomy, in either young or adult birds. On the other hand, adrenal weight was reduced after hypophysectomy in young quail. A decrease in adrenal weight and no change in thyroid weight was also reported by Nagra _et al_ (1963) for the male pheasant (operated at 8 weeks of age). In ducks, a significant decrease of adrenal weight was also reported in young birds (Bradley and Holmes, 1971) and in adult birds (Boissin _et al_, 1966). In the pigeon, adrenal weight was considerably reduced 22 days after hypophysectomy in young birds (1.9 to 2.4 months old), but there was no significant difference 5 days after the operation (Miller, 1961).

On the contrary, 7 - 12 week old male chickens did not show a reduction in adrenal weight after hypophysectomy (Mitchell, 1929; Baum and Meyer, 1956; Newcomer, 1959; Nagra _et al_, 1963), while thyroid weight was reduced considerably after hypophysectomy (Mitchell, 1929; Baum and Meyer, 1956; Nagra _et al_, 1963). In adult hens, the thyroid showed considerable atrophy 27 days after hypophysectomy (Mitchell, 1970). However, in chicks (3 week old males), a considerable reduction of adrenal weight was observed 22

days after hypophysectomy (King, 1969). This is in agreement with the finding that the adrenal was more sensitive to ACTH in 3 - 4 week old chicks than in older birds (Siegel, 1962). Ma and Nalbandov (1963) reported that in growing chickens (age is not given) adrenal weight of hypophysectomized birds was lower than normal until 20 days after the operation, but that there was no significant difference from 20 days to 60 days after hypophysectomy.

These results for the chicken, together with the effects of stress on the adrenal glands of hypophysectomized animals (Brown _et al_, 1959), have led several investigators to propose an autonomous function of the avian adrenal, or else an extrapituitary origin of ACTH in birds (Brown _et al_, 1958; Newcomer, 1959; Ma and Nalbandov, 1963). However, Bradley and Holmes (1971) have rejected this idea because of their results in ducks. Thus, no general statement can be made regarding the influence of the pituitary on the thyroid and adrenal glands of birds. Since the adult chicken's response was opposite to that of most other avian species studied, and since young chicks responded differently to hypophysectomy than adults as far as thyroid and adrenal weights were concerned, species and age differences may be more important among birds than mammals.

Molting was observed in all hypophysectomized birds. This confirms the report by Hill and Parkes (1935)

for the chicken. According to Tanabe et al (1957) molting in the intact hen is not induced by a change in thyroid activity, but by a decrease in ovarian activity. This hypothesis fits the results reported here which showed considerable reduction of gonads and conspicuous molting after hypophysectomy, but no change in thyroid weight. Molting was also observed when Japanese quail were transferred to a short photoperiod (Oishi, unpublished). This might also be due to the considerable atrophy of the gonads induced by the short photoperiod.

D. ULTRASTRUCTURE OF THE PINEAL BODY UNDER LIGHT AND DARK CONDITIONS

INTRODUCTION

The effects of the photoperiod on the amount of indoleamines and on enzyme activity (hydroxyindole-O-methyltransferase: HIOMT) in the pineal body of birds are well known (Axelrod et al, 1964; Quay, 1966; Ralph et al, 1967; Lauber et al, 1968; Alexander et al, 1970 a, b; Barfuss and Ellis, 1971; Lynch, 1971; see also Section IV-B). A photoreceptor function by the avian pineal is suggested by several authors. Rosner et al (1971) showed that light increased HIOMT activity of the duck pineal in vitro. Munns (1970) reported that light caused high pineal HIOMT activity and increased testis weight in the canary, while covering of the pineal by black polyester resin abolished this response. Oishi and Kato (1968) also showed that direct illumination of the pineal with radioluminescent paint led to maintenance of large testes in Japanese quail. Pinealectomy abolished the photoperiodic response of the pituitary and the adrenal (see Section IV-A). On the other hand, several electrophysiological studies failed to show any response of the pineal to illumination (Morita, 1966 b in the pigeon; Ralph and Dawson, 1968 in Japanese quail and the house sparrow; Oksche et al, 1969 in the

pigeon).

Quay and Renzoni (1963, 1966) reported that lengthening of the photoperiod induced an increase in nuclear size of pineal parenchymal cells and in the number of stainable commissuropineal neurosecretory cells in the house sparrow. On the other hand, Ralph and Lane (1969), in studies on the house sparrow, and Lane et al (1969) in Japanese quail, reported no correlation between changes in pineal cytology and the photoperiod, age or stage of sexual maturation.

Ultrastructural studies have revealed that the avian pineal has cells with a lamellar complex attached to cilia. These have been considered by some investigators to be rudimentary photoreceptor structures (Oksche and Kirschstein, 1969, in the house sparrow; Collin, 1971, in the magpie).

A secretory function by the avian pineal is suggested by the existence of secretory granules in the parenchymal cells (Fujie, 1968; Quay et al, 1968; Bischoff, 1969; Oksche and Kirschstein, 1969; Ueck, 1970; Collin, 1971). However, there is only one report which suggests a relationship between the photoperiod and avian pineal ultrastructure. Fujie (1968) reported a greater amount of cytoplasm, which contained an increased number of small

granules, lysosomes and lipid droplets, and a decreased number of dense-cored synaptic vesicles, in birds subjected to a long photoperiod.

Consequently, pineal ultrastructure was studied in Japanese quail exposed to continuous light or continuous darkness in order to determine the effects of the photoperiod on pineal cytology. The pineal body of newly hatched quail was also examined.

MATERIALS AND METHODS

Male Japanese quail, reared under continuous light until 17 weeks of age, were divided into two groups. Ten birds of one group were kept in continuous light and 10 others were kept in continuous darkness for one week. In addition, two small groups of 10.5 week old male quail reared under continuous light were assigned as follows: one group (3 birds) in continuous light and the other group (2 birds) in continuous darkness, for 5 weeks. Pineals were also taken from newly hatched quail (9 birds).

Birds were killed by decapitation in the light or dark conditions to which they had been assigned, and the pineal body was immediately removed and fixed in 3% glutaraldehyde in 0.25 M sucrose - 0.1 M phosphate buffer (pH 7.4) (Sabatini *et al*, 1964) for at least 2 hours and then postfixed in 1% osmium tetroxide in 0.1 M phosphate

buffer (pH 7.4) for 2 hours. After fixation, the specimens were dehydrated with alcohol, infiltrated with propylene oxide, and embedded in Epon 812. Sections were made with a Porter-Blum MT 2 ultramicrotome using glass knives, and were doubly stained with 5% uranyl acetate in methyl alcohol, and lead citrate (Reynolds, 1963). Specimens were observed with a Phillips EM 200 electron microscope.

RESULTS

The button-like distal end of the quail pineal body consists of many lobules. In each lobule, parenchymal cells are situated around a central lumen, which is surrounded in turn by connective tissue, capillaries and nerve fibers. Three types of cells were identified in the parenchyma: photoreceptor cells (pinealocytes), supportive cells (glial cells), and nerve cells (ganglion cells).

Photoreceptor cell (pinealocyte): This type of cell has a cilium and microvilli extending into the lumen (Figs. 25, 26, 33 and 34). Each cilium is connected to a whorl of lamellar complex (Fig. 32) which is considered to be a degenerated organelle comparable to the outer segment of the retina. In one case from a newly hatched quail pineal, a disc-like arrangement of the lamellae was observed (Fig. 25). The microtubules of the cilium usually show a (9 + 0) configuration (Fig. 27), or sometimes a (9 + 2) configu-

ration (Fig. 32). The cilium is connected to a cell process protruding into the lumen of the lobule ("inner segment" of Figs. 25, 26, 27 and 34), which is rich in mitochondria. This inner segment in the newly hatched quail pineal displays an abundant Golgi apparatus, as well as both clear and dense-cored vesicles (Fig. 26). The inner segment is separated from the rest of the cell by a necklike constriction, and at this portion cell junctions (zonula adherens) can be seen between the neighbouring cells (Figs. 27, 34). In this area, numerous microtubules run parallel to the long axis of the cell (Fig. 27). The nucleus is irregular in shape with one or two prominent nucleoli and is situated in the basal portion of the cell. In the cytoplasm at the proximal part of process, there are endoplasmic reticulum, scattered ribosomes, mitochondria, and Golgi apparatus associated with numerous vesicles (Figs. 28, 35). The basal portion of the photoreceptor cell extends many processes toward the basement membrane (Figs. 29, 30, 31, 36), these processes intermingling with nerve fibers. Occasionally, the end of such a process can be seen in the pericapillary area. Vesicle-crowned synaptic ribbons are occasionally observed in the process close to the basement membrane (Figs. 40, 47). Where a nerve ending is surrounded by processes of photoreceptor cells,

desmosome-like cell junctions can be observed between the nerve ending and the photoreceptor cell process (Fig. 42).

Supportive cell (glial cell): This cell type shows clearer cytoplasm than the pinealocyte, with more abundant organelles (Figs. 27, 28, 34). Many microvilli line the apical surface, but the cell lacks cilia. Numerous mitochondria, Golgi apparatus, endoplasmic reticulum, scattered ribosomes and microtubules can be seen in the cytoplasm. The processes of this cell are difficult to distinguish from the processes of nerve cells.

Nerve cell (Ganglion cell): The nerve cell body contains a nucleus, numerous mitochondria, and prominent endoplasmic reticulum. The perikaryon is embedded in the processes of photoreceptor cells close to the basement membrane (Figs. 30, 36). Figure 36 shows several nerve processes which are considered to be dendrite and axon. Each contains numerous filaments. The nerve ending contains numerous synaptic vesicles (about 500 Å in diameter) and dense-cored vesicles of larger size (1,000 Å in diameter) (Figs. 29, 31, 42, 45, 49, 50).

Interlobular connective tissue: Red blood cells are seen in the capillaries, which are lined by fenestrated endothelial cells (Figs. 45, 48). Unmyelinated nerve bundles are embedded in collagen fibers in the pericapillary area

(Figs. 37, 48). Myelinated nerve fibers are also seen in the pericapillary area (Fig. 37).

The pineal photoreceptor cell from quail kept in continuous light: Close to the basement membrane, the photoreceptor cell processes contain many dense-cored, membrane-limited secretory granules (800 - 1,200 Å in diameter) (Figs. 38, 39, 40, 41, 42). Nearby are nerve processes containing synaptic vesicles and dense-cored membrane-limited vesicles (800 - 1,100 Å in diameter). Occasionally a half-depleted dense-cored vesicle is seen (Fig. 41).

The pineal photoreceptor cell from quail held in continuous darkness for 1 week: Membrane-limited, dense-cored vesicles (1,000 - 1,300 Å in diameter) are observed in the processes close to the lobular lumen (Figs. 43, 44). This could not be observed in the pineal of animals in continuous light. In the pericapillary region, the processes of the photoreceptor cell have only a few secretory granules (Fig. 45), as contrasted with the situation in continuous light. In the nerve endings, numerous synaptic vesicles and some dense-cored, membrane-limited granules are observed as in the case of birds kept in continuous light.

The pineal photoreceptor cell from quail kept in continuous darkness for 5 weeks: Membrane-limited, dense-cored vesicles are found in the processes close to the lumen

(Fig. 46), as in the pineal of birds in darkness for 1 week. Secretory granules could not be found in the processes close to the basement membrane (Figs. 47, 48, 49). In the nerve endings, membrane-limited vesicles are observed, and most of these show half or complete depletion (Figs. 49, 50).

DISCUSSION

Although there is physiological evidence indicating a photoreceptor function of the avian pineal, "typical" photoreceptor structures could not be observed except in one case in a newly hatched quail. It should also be noted that the brain of birds (probably the hypothalamus), which has not been reported to have a specific photoreceptor structure, seems to receive light (Benoit, 1964; Homma, 1969, Menaker, 1971; see also Section III-A).

The finding of both secretory and (apparently) sensory structures in the same cell type (secretory and rudimentary photoreceptor cell - Collin, 1971) is confirmed. More secretory granules are seen in the pineals of birds in continuous light than in continuous darkness, in the photoreceptor cell processes of the pericapillary region. This suggests that the secretory activity of the pineal of Japanese quail is quite high in continuous light and low in continuous darkness, which confirms the report on the

chicken by Fujie (1968) and coincides with the findings on enzyme activity in light and darkness (see Section IV-B). However, some secretory granules of almost the same size as those described above were observed in the photoreceptor cell processes close to the lumen when the birds were kept in continuous darkness, and secretory granules in this location could not be observed in the pineal in continuous light. This finding suggests that secretion into the capillaries occurs under light conditions, and into the lumen in darkness. Identification of the substances within these granules remains for further investigation.

In nerve endings in the parenchyma, completely depleted or half-depleted membrane-limited vesicles were observed more frequently in darkness than in light. Since the origin of these nerve endings was hard to identify, it is not clear whether they are from sympathetic nerve fibers or from ganglion cells in the parenchyma. However, since sympathetic nerve fibers perforating the basement membrane into the parenchyma could not be observed in the present study, as reported in the sparrow by Ueck (1970), it seems more likely that these nerve endings originate from ganglion cells.

The contact between processes of the photoreceptor cells and nerve endings, presented in this study, which confirms the report in the sparrow by Ueck (1970), suggests

the existence of "type one" cells (photoreceptor cell in connection with a ganglion cell, as classified by Collin, 1971) in the avian pineal.

V. GENERAL DISCUSSION

The gonads of both male and female Japanese quail respond remarkably to the photoperiod. This response was observed in the maintenance of gonadal weight by adult birds, as well as in the developmental phase. However, since Farner and Follett (1966) reported that the gonadal growth curve is not the same for young as for adult quail, the possibility must be considered that different mechanisms are involved in the photoperiodic gonadal response during the two phases. The response of the adrenal to the photoperiod was also different in young than in adult animals (Section IV-A). It follows that one must consider not only the effect of light on tropic hormone production and release, and thus on maintenance of target organs, but also the effect of light on development of the hypothalamico-hypophyseal-target organ system, on the development of the sensitivity of each endocrine organ to its tropic hormone, and on the interrelationships of the several endocrine organs. The maintenance phase of gonadal activity thus seems to offer an ideal system for the study of photoperiodic gonadal responses, excluding the effects of light on the developing gonads via other systems described above.

The pineal body seems to be involved in the developmental phase of the photo-endocrine response. The adrenal

and the pituitary of young quail responded to the photoperiod and this response was abolished by pinealectomy (Section IV-A). Hypophysectomy reduced adrenal weight of young quail considerably, showing a pituitary-adrenal relationship (Section IV-C). Singh and Turner (1967) reported that the administration of melatonin induced a decrease of adrenal weight in the chicken. These findings together with the marked effect of the photoperiod on pineal enzymes and on pineal ultrastructure (Section IV-B, D) suggest an important role of the pineal in the photoperiodic responses and in the pituitary-adrenal axis relationship. On the other hand, thyroid weight was not affected either by the photoperiod or by pinealectomy. Even hypophysectomy could not induce any change in thyroid weight (Section IV-C). Singh and Turner (1967) reported no effect of melatonin on thyroid weight and on the thyroid secretion rate in chickens. Thus, the avian thyroid appears to be autonomous at least in the species so far examined (see Section IV-C). Singh and Turner (1967) further reported a marked effect of melatonin on thymus weight. It would be of interest to investigate the effect of the photoperiod and of pinealectomy on the thymus.

Since Hamner (1963) demonstrated the involvement of the biological clock in the photoperiodic gonadal response

of birds, the importance of the clock in this system has been noted by several other investigators (Wolfson, 1965; Farner and Follett, 1966; Follett and Sharp, 1969). Menaker and his colleagues recently reported evidence for pineal-mediated entrainment of locomotor activity rhythm and body temperature rhythm in the house sparrow (Gaston and Menaker, 1968; Binkley et al, 1971; Menaker, 1971). These reports suggest the possibility that the pineal acts as a "clock center" which regulates the photoperiodic gonadal response and other photoendocrine responses. The present results (Section IV-B, D) show pronounced effects of the light-dark cycle on pineal enzyme activity and on pineal ultrastructure, and provide supporting evidence for the proposed role of the pineal as a "clock center". The interrelationships between the photoreceptor(s), the biological clock and the hypothalamo - endocrine organ systems, and the role of the pineal in this system are interesting problems for later investigation.

The results of Section III indicate that an extraretinal, extrapineal, extra Harderian gland photoreceptor is the major photoreceptor for light-induced gonadal maintenance. However, the pineal seems to be the photoreceptor for the photoperiodic adrenal response. This suggests different photoreceptor systems for the several photobiological phenomena and confirms Menaker's (1971) finding

that photoperiodically-induced gonadal activity and entrainment of locomotor activity rhythm are separately controlled. The threshold intensity and the action spectrum under low light intensity for the maintenance of mature testes of quail were determined to be almost the same in blinded birds as in intact birds. Thus, the extraretinal photoreceptor is shown to be highly sensitive to light, although it does not seem to have specific structures for photoreception. The precise location of this receptor and its mechanism of photoreception remain to be explored in future experiments.

VI. CONCLUSIONS

1. The photoreceptor(s) for light-induced gonadal maintenance in male Japanese quail was shown to be restricted to the head region.

2. The primary photoreceptor was shown to be at an extraretinal, extrapineal, extra Harderian gland site, probably in the brain itself.

3. The eyes and the pineal body appear to function as auxiliary photoreceptors or as light guides. There is no evidence that the Harderian gland serves as a photoreceptor in Japanese quail.

4. The light intensity threshold necessary to maintain maximal gonadal size in both intact and enucleated adult male quail was determined to be approximately 1.57 - 1.67 $\mu w/cm^2$.

5. In intact birds, the portions of the visible spectrum effective for maintenance of full gonadal maturity were red (625 nm) and green (500 nm) when the light was ten times the threshold level (dim light), while only red light was effective at the threshold level (x 0.1 dim light).

6. In enucleated birds, the action spectrum under dim light was the same as for intact birds under x 0.1 dim light.

7. Weights of developing endocrine organs (pituitary, adrenal and gonad) were higher in continuous light (24L/0D) than in a short photoperiod (8L/16D) in 5-week old Japanese quail after 3 weeks of lighting treatment. These light-induced responses of pituitary weight and adrenal weight were abolished by pinealectomy in both males and females, while gonadal weight was not affected by pinealectomy. There was no effect of either the photoperiod or of pinealectomy on body weight or thyroid weight.

8. Hypophysectomy caused marked atrophy of the gonads (young females and adults of both sexes) and adrenals (6.5 week old females and 10 week old males) within 10 - 14 days. Body weight, thyroid weight, pineal weight and pineal hydroxyindole-O-methyltransferase (HIOMT) activity were not altered by hypophysectomy.

9. Pineal HIOMT activity of adult birds was high in light and low in darkness. The optimum temperature for HIOMT activity assay _in vitro_ was determined to be 47 °C.

10. Three types of cells were identified electron microscopically in the parenchyma of the pineal: photoreceptor cells (pinealocytes), supportive cells (glial cells), and nerve cells (ganglion cells). Both rudimentary photoreceptor structures and secretory granules (800 - 1,200 Å in diameter) were observed in the photoreceptor

cells. There was a greater number of secretory granules in the photoreceptor cell processes close to the pericapillary area under continuous light than under continuous darkness. Under continuous darkness, the photoreceptor cell processes contained secretory granules close to the lobular lumen. Half-depleted and completely depleted dense-cored membrane-limited vesicles were frequently observed in nerve endings under continuous darkness.

VII. REFERENCES CITED

Adler, K. 1969. Extraoptic phase shifting of circadian locomotor rhythm in salamanders. Science 164: 1290-1292.

Adler, K. 1970. The role of extroptic photoreceptors in amphibian rhythms and orientation: a review. J. Herpetol. 4: 99-112.

Aho, W. A., W. O. Wilson, and T. D. Siopes. 1970. Effect of brooding with 2.3 and 3.0 micron wavelength radiant heat sources on gonadal weights in 5-week old coturnix. Poultry Sci. 49: 369-371.

Alexander, B., A. J. Dowd, and A. Wolfson. 1970 a. Pineal hydroxyindole-O-methyltransferase (HIOMT) activity in female Japanese quail (Coturnix coturnix japonica). Neuroendocrinol. 6: 236-245.

Alexander, B., A. J. Dowd, and A. Wolfson. 1970 b. Effect of continuous light and darkness on hydroxyindole-O-methyltransferase and 5-hydroxytryptophan decarboxylase activities in the Japanese quail. Endocrinol. 86: 1441-1443.

Allardyce, J., J. Aldous, W. Cooper, J. Pratt, and E. Sutherland. 1942. Effects of visible radiations upon albino rats. Am. J. Physiol. 137: 761-768.

Arrington, L. C., R. K. Ringer, and J. H. Wolford. 1969. Effect of pinealectomy of Coturnix quail reared under non-stimulatory photoperiods. Poultry Sci. 48: 454-459.

Asmundson, V. S., F. W. Lorenz, and B. D. Moses. 1946. Influence of light intensity on ovulation in turkeys. Poultry Sci. 25: 346-354.

Asmundson, V. S., F. H. Kratzer, and B. D. Moses. 1951. Relation of all-night light to egg quality in turkeys. Poultry Sci. 30: 546-548.

Axelrod, J., and J. K. Lauber. 1968. Hydroxyindole-O-methyltransferase in several avian species. Biochem. Pharmacol. 17: 828-830.

Axelrod, J., R. J. Wurtman, and C. M. Winget. 1964. Melatonin synthesis in the hen pineal gland and its control by light. Nature 201: 1134.

Axelrod, J., P. D. MacLean, R. W. Albers, and H. Weissbach. 1961. Regional distribution of methyl transferase enzymes in the nervous system and glandular tissues. In: Regional Neurochemistry, S. S. Kety and J. Elkes, ed. Pergamon Press, Oxford. pp. 307-311.

Barbanti-Silva, E. 1932. Influenza di alcune luci colorate sulle funzioni della riproduzione e dell'accrescimento. Monit. Ostet. Gin. 4: 145-155.

Barfuss, D. W., and L. C. Ellis. 1971. Seasonal cycles in melatonin synthesis by the pineal gland as related to testicular function in the house sparrow (Passer domesticus). Gen. Comp. Endocrinol. 17: 183-193.

Bartholomew, G. A. 1949. The effect of light intensity and day length on reproduction in the English sparrow. Bull. Museum Comp. Zool. Harvard Univ. 101: 433-476.

Basrur, P. K., and C. M. Winget. 1963. Histological studies on the pineal body of normal and light treated birds. Poultry Sci. 42: 1255.

Baum, G. J., and R. K. Meyer. 1956. Influence of diethylstilbestrol on lipids in intact and hypophysectomized cockerels. Endocrinol. 58: 338-346.

Baumann, F., A. Mauro, R. Milecchia, S. Nightingale, and J. Z. Young. 1970. The extra-ocular light receptors of the squids Todarodes and Illex. Brain Research 21: 275-279.

Baylé, J. D., M. Kraus, and A. Van Tienhoven. 1970. The effects of hypophysectomy and testosterone propionate on the testes of Japanese quail, Coturnix coturnix japonica. J. Endocrinol. 46: 403-404.

Benoit, J. 1935 a. Rôle des yeux dans l'action stimulante de la lumière sur le développement testiculaire chez le canard. C. R. Soc. Biol. 118: 669-671.

Benoit, J. 1935 b. Stimulation par la lumière artificielle du développement testiculaire chez des canards aveuglés par section du nerf optique. C. R. Soc. Biol. 120: 133-136.

Benoit, J. 1935 c. Stimulation par la lumière artificielle du développement testiculaire chez des canards aveuglés par énucléation des globes oculaires. C. R. Soc. Biol. 120: 136-139.

Benoit, J. 1937. Facteurs externes et internes de l'activité sexuelle. II. Etude du mécanisme de la stimulation par la lumière de l'activité testiculaire chez le canard domestique. Rôle de l'hypophyse. Bull. Biol. France Belgique 72: 394-437.

Benoit, J. 1964. The role of the eye and of the hypothalamus in the photostimulation of gonads in the duck. In: Photo-Neuro-Endocrine Effects in Circadian Systems, with Particular Reference to the Eye. H. E. Whipple, ed. Annals of the New York Academy of Sciences 117: 204-216.

Benoit, J., and I. Assenmacher. 1966. Recherches sur la photosensibilité des récepteurs nerveux superficiel et profond dans la gonadostimulation par les radiations visibles chez le canard Pékin impubère. C. R. Acad. Sci. Paris 262: 2750-2752.

Benoit, J., I. Assenmacher, and F. X. Walter. 1952. Différences de sensibilité de la rétine du canard aux radiations colorées dans le réflexe pupillaire et dans le réflexe opto-sexuel. C. R. Soc. Biol. 146: 1027-1030.

Benoit, J., C. Da Lage, B. Muel, C. Kordon, and I. Assenmacher. 1966. Localisation dans le spectre visible de la zone de sensibilité rétinienne maximale aux radiations lumineuses impliquées dans la gonadostimulation chez le canard Pékin impubère. C. R. Acad. Sci. Paris 263: 62-64.

Bick, R. L., R. A. Giolli, L. C. Dearden, and R. R. Stuart. 1969. The effect of pinealectomy and environmental lighting on the gonadal, thyroid, and total body weight of female Long-Evans rats. Experientia 25: 531-532.

Binkley, S., E. Kluth, and M. Menaker. 1971. Pineal function in sparrows: Circadian rhythms and body temperature. Science 174: 311-314.

Bischoff, M. B. 1969. Photoreceptoral and secretory structures in the avian pineal organ. Journal of Ultrastructure Research 28: 16-26.

Bissonnette, T. H. 1931 a. Studies on the sexual cycle in birds. IV. Experimental modification of the sexual cycle in males of the European starling (Sturnus vulgaris) by changes in the daily period of illumination and of muscular work. J. Exp. Zool. 58: 281-318.

Bissonnette, T. H. 1931 b. Studies on the sexual cycle in birds. V. Effects of light of different intensities upon the testis activity of the European starling (Sturnus vulgaris). Physiol. Zool. 4: 542-574.

Bissonnette, T. H. 1932. Studies on the sexual cycle in birds. VI. Effects of white, green, and red lights of equal luminous intensity on the testis activity of the European starling (Sturnus vulgaris). Physiol. Zool. 5: 92-123.

Bissonnette, T. H. 1935. Modification of mammalian sexual cycles. IV. Delay of oestrus and induction of anoestrus in female ferrets by reduction of intensity and duration of daily light periods in the normal oestrus season. J. Exp. Biol. 12: 315-320.

Boissin, J., J. D. Baylé, and I Assenmacher. 1966. Le fonctionnement cortico-surrénalien du canard mâle après préhypophysectomie ou autogreffe hypophysaire ectopique. C. R. Acad. Sci. Paris 263: 1127-1129.

Bradley, E. L., and W. N. Holmes. 1971. The effects of hypophysectomy on adrenocortical function in the duck (Anas platyrhynchos). J. Endocrinol. 49: 437-457.

Brown, K. I., D. J. Brown, and R. K. Meyer. 1958. Effect of surgical trauma, ACTH and adrenal cortical hormones on electrolytes, water balance and gluconeogenesis in male chickens. Am. J. Physiol. 192: 43-50.

Burger, J. W. 1939. Some aspects of the roles of light intensity and the daily length of exposure to light in the sexual photoperiodic activation of the male starling. J. Exp. Zool. 81: 333-341.

Burger, J. W. 1943. Some effects of colored illumination on the sexual activation of the male starling. J. Exp. Zool. 94: 161-168.

Carson, J. R., W. A. Junnila, and B. F. Bacon. 1958. Sexual maturity and productivity in the chicken as affected by the quality of illumination during the growing period. Poultry Sci. 37: 102-112.

Clark, L. B., S. L. Leonard, and G. Bump. 1937. Light and the sexual cycle of game birds. Science 85: 339-340.

Clausen, H. J., and B. Mofshin. 1939. The pineal eye of the lizard (Anolis carolinensis), a photoreceptor as revealed by oxygen consumption studies. J. Cell. Comp. Physiol. 14: 29-41.

Collin, J. P. 1971. Differentiation and regression of the cells of the sensory line in the epiphysis cerebri. In: The Pineal Gland. G. E. W. Wolstenholme and J. Knight, ed. Churchill Livingstone, Edinburgh and London. pp. 79-120.

Critchlow, V. 1963. The role of light in the neuro-endocrine system. In: Advances in Neuroendocrinology. A. V. Nalbandov, ed. Univ. of Illinois Press, Urbana, Illinois. pp. 377-402.

Dakan, E. L. 1934. Light absorption, feed consumption, and the urge to lay. Poultry Sci. 13: 317-318.

De Wilde, J., C. S. Duintjer, and L. Mook. 1959. Physiology of diapause in the adult Colorado beetle (Leptinotarsa decemlineata Say). I. The photoperiod as a controlling factor. J. Ins. Physiol. 3: 75-85.

Derrien, E., and J. Turchini. 1924. Sur l'accumulation d'une porphyrine dans la glande de Harder des Rongeurs du genre Mus et sur son mode d'excrétion. C. R. Soc. Biol. 91: 637-639.

Dodt, E. 1963. Photosensitivity of the pineal organ in the teleost, Salmo irideus (Gibbons). Experientia 19: 642-643.

Dodt, E., and E. Heerd. 1962. Mode of action of pineal nerve fibers in frogs. J. Neurophysiol. 25: 405-429.

Dodt, E., and M. Jacobson. 1963. Photosensitivity of a localized region of the frog diencephalon. J. Neurophysiol. 26: 752-758.

Dodt, E., and Y. Morita. 1967. Conduction of nerve impulses within the pineal system of the frog. Pflügers Archiv 293: 184-192.

Dodt, E., and E. Scherer. 1968. Photic responses from the parietal eye of the lizard Lacerta sicula campestris (De Betta). Vision Res. 8: 61-72.

Dorminey, R. W., J. E. Parker, and W. H. McClusky. 1970. Effects of light intensity on Leghorn pullets during the development and laying periods. Poultry Sci. 49: 1657-1661.

Eddy, J. M. P., and R. Strahan. 1968. The role of the pineal complex in the pigmentary effector system of Geotria australis (Gray). Gen. Comp. Endocrinol. 11: 528-534.

Farner, D. S. 1959. Photoperiodic control of annual gonadal cycles in birds. In Photoperiodism and Related Phenomena in Plants and Animals R. B. Withrow, ed. American Association for the Advancement of Science, Washington, D. C. pp. 717-750.

Farner, D. S. 1970. Some glimpses of comparative avian physiology. Fed. Amer. Soc. Exp. Biol. Fed. Proc. 29: 1649-1663.

Franer, D. S., and B. K. Follett. 1966. Light and other environmental factors affecting avian reproduction. J. Animal Sci. 25: 90-118.

Fenwick, J. C. 1970. Effects of pinealectomy and bilateral enucleation on the phototactic response and on the conditioned response to light of the goldfish Carassius auratus L. Can. J. Zool. 48: 175-182.

Fiske, V. M., and H. H. Lambert. 1962. Effect of light on the weight of the adrenal in the rat. Endocrinol. 71: 667-668.

Follett, B. K., and P. J. Sharp. 1969. Circadian rhythmicity in photoperiodically induced gonadotrophin release and gonadal growth in the quail. Nature 223: 968-971.

Fujie, E. 1968. Ultrastructure of the pineal body of the domestic chicken, with special reference to the changes induced by altered photoperiods. Arch. Histol. Jap. 29: 271-303.

Ganong, W. F., M. D. Shepherd, J. R. Wall, E. E. Van Brunt, and M. T. Clegg. 1963. Penetration of light into the brain of mammals. Endocrinol. 72: 962-963.

Gaston, S., and M. Menaker. 1968. Pineal function: The biological clock in the sparrow? Science 160: 1125-1127.

Gorbman, A., and H. A. Bern. 1966. A Textbook of Comparative Endocrinology. John Wiley and Sons, Inc., New York. London. Sydney.

Grafflin, A. L. 1942. Histological observations upon the porphyrin-excreting Harderian gland of the albino rat. Am. J. Anat. 71: 43-64.

Hafeez, M. A., and W. B. Quay. 1970. The role of the pineal organ in the control of phototaxis and body coloration in rainbow trout (Salmo gairdneri, Richardson). Zeit. Vergleich. Physiol. 68: 403-416.

Hamasaki, D. I. 1968. Properties of the parietal eye of the green iguana. Vision Res. 8: 591-599.

Hamner, W. M. 1963. Diurnal rhythm and photoperiodism in testicular recrudescence of the house finch. Science 142: 1294-1295.

Hanyu, I., H. Niwa, and T. Tamura. 1969. A slow potential from the epiphysis cerebri of fishes. Vision Res. 9: 621-623.

Harrison, P. C., and W. C. Becker. 1969. Extraretinal photocontrol of oviposition in pinealectomized domestic fowl. Proc. Soc. Exp. Biol. Med. 132: 161-164.

Harrison, P. C., J. McGinnis, G. Schumaier, and J. Lauber. 1969. Sexual maturity and subsequent reproductive performance of White Leghorn chickens subjected to different parts of the light spectrum. Poultry Sci. 48: 878-883.

Harrison, P. C., J. D. Latshaw, J. M. Casey, and J. McGinnis. 1970. Influence of decreased length of different spectral photoperiods on testis development of domestic fowl. J. Reprod. Fert. 22: 269-275.

Hedlund, L., C. L. Ralph, J. Chepko, and H. J. Lynch. 1971. A diurnal serotonin cycle in the pineal body of Japanese quail: Photoperiod phasing and the effect of superior cervical ganglionectomy. Gen. Comp. Endocrinol. 16: 52-58.

Hill, R. T., and A. S. Parkes. 1934. Hypophysectomy of birds. I. Technique, with a note on results. Proc. Roy. Soc. London, Series B. 115: 402-409.

Hill, R. T., and A. S. Parkes. 1935. Hypophysectomy of birds. V. Effect of replacement therapy on the gonads, accessory organs and secondary sexual characters of hypophysectomized fowls. Proc. Roy. Soc. London, Series B. 117: 210-218.

Hollwich, F., and S. Tilgner. 1961 a. Experimentelle Untersuchungen über den Einfluss monochromatischen Lichtes auf die Hodenentwicklung des Erpels. Klin. Monatsbl. Augenheilk. 139: 828-835.

Hollwich, F., and S. Tilgner. 1961 b. Experimentelle Untersuchungen über den photosexuellen Reflex (réflexe opto-sexuel) bei der Ente. Ophthalmologica 142: 572-576.

Homma, K. 1969. Gonadal development in the Japanese quail after local photic stimulation to the brain. In: Seminar on Hypothalamic and Endocrine Function in Birds. Int. House of Japan, Tokyo. pp. 56-57.

Homma K., L. Z. McFarland, and W. O. Wilson. 1967. Response of the reproductive organs of the Japanese quail to pinealectomy and melatonin injections. Poultry Sci. 46: 314-319.

Houssay, A. B., and J. H. Pazo. 1968. Role of pituitary in the thyroid hypertrophy of pinealectomized rats. Experientia 24: 813-814.

Ishibashi, T. 1957. Studies on the effects of the artificial illumination upon the development of the gonad in the fowl. VI. Influences of removal of the bulbo oculi on the gonad. Sci. Rep. Hyogo Univ. Agric. 3: 27-30.

Ishibashi, T., and Y. Kato. 1951. Effects of irradiation with different wavelengths on the development of the gonad in the fowl. Sci. Bull. Fac. Agri. Kyushu Univ. 13: 392-395.

Ivanova, S. 1935. Über den Mechanismus der Wirkung von Licht auf die Hoden der Vögel (Passer domesticus). Arch. Exp. Path. Pharmak. 179: 349-359.

Johnson, G. E., and E. L. Gann. 1933. Light in relation to the sexual cycle and to hibernation in the thirteen-lined ground squirrel. Anat. Rec. 57: Suppl. pp. 28.

Kannankeril, J. V. 1970. Effect of pinealectomy on the hypertrophy of the rudimentary right gonad following sinistral ovariectomy in the Japanese quail. (Coturnix coturnix japonica). Anat. Rec. 166: 328.

Karasek, M. 1971. The influence of hypophysectomy on the ultrastructure of the pineal gland in white rats. Acta Med. Pol. 12: 153-156.

Kennedy, D. 1961. Neural photoreception in a lamellibranch mollusc. J. Gen. Physiol. 44: 277-299.

Kennedy, D. 1963. Physiology of photoreceptor neurons in the abdominal nerve cord of the crayfish. J. Gen. Physiol. 46: 551-572.

King, D. B. 1969. Effect of hypophysectomy on the radioactive phosphorus uptake of chick adrenals. Poultry Sci. 48: 459-464.

Kirkpatrick, C. M. 1955. Factors in photoperiodism of bobwhite quail. Physiol. Zool. 28: 255-264.

Kitay, J. I., and M. D. Altschule. 1954. The Pineal Gland--A review of the physiologic literature. Harvard University Press, Cambridge, Mass.

Kleinpeter, M. E., and J. P. Mixner. 1947. The effect of the quantity and quality of light on the thyroid activity of the baby chick. Poultry Sci. 26: 494-498.

Klüver, H. 1944. Porphyrins, the nervous system, and behavior. J. Psychology 17: 209-227.

Lane, K. B., C. L. Ralph, and S. Gilbert. 1969. Lack of correlation between sexual maturation and pineal cytology in the Japanese quail. Anat. Rec. 163: 215

Lauber, J. K., J. E. Boyd, and J. Axelrod. 1968. Enzymatic synthesis of melatonin in avian pineal body: Extraretinal response to light. Science 161: 489-490.

Lees, A. D. 1964. The location of the photoperiodic receptors in the aphid Megoura viciae Buckton. J. Exp. Biol. 41: 119-133.

Lisk, R. D., and L. R. Kannwischer. 1964. Light: Evidence for its direct effect on hypothalamic neurons. Science 146: 272-273.

Luce-Clausen, E. M., and E. F. Brown. 1939. The use of isolated radiation in experiments with the rat. III. Effects of darkness, visible and infra-red radiation on three succeeding generations of rats (b) Reproduction. J. Nutrition 18: 551-562.

Lupulescu, A. 1968. Ultrastructure of the pineal gland after hypophysectomy. Experientia 24: 482-484.

Lynch, H. J. 1971. Diurnal oscillations in pineal melatonin content. Life Sci. 10: Part I 791-795.

Ma, R. C. S., and A. V. Nalbandov. 1963. Discussion In Advances in Neuroendocrinology. A. V. Nalbandov, ed., University of Illinois Press, Urbana, Illinois. pp. 306-313.

Marshall, F. H. A. 1940. The experimental modification of the oestrus cycle in the ferret by different intensities of light irradiation and other methods. J. Exp. Biol. 17: 139-146.

Marshall, F. H. A., and F. P. Bowden. 1934. The effect of irradiation with different wave-lengths on the oestrous cycle of the ferret, with remarks on the factors controlling sexual periodicity. J. Exp. Biol. 11: 409-422.

Marshall, F. H. A., and F. P. Bowden. 1936. The further effects of irradiation on the oestrous cycle of the ferret. J. Exp. Biol. 13: 383-386.

McFarland, L. Z., K. Homma, and W. O. Wilson. 1968. Superior cervical ganglionectomy in the Japanese quail. Experientia 24: 245-247.

Menaker, M. 1968. Extraretinal light perception in the sparrow, I. Entrainment of the biological clock. Proc. Nat. Aca. Sci. U. S. A. 59: 414-421.

Menaker, M. 1971. Rhythms, reproduction, and photoreception. Biol. Reprod. 4: 295-308.

Menaker, M., and H. Keatts. 1968. Extraretinal light perception in the sparrow, II. Photoperiodic stimulation of testis growth. Proc. Nat. Acad. Sci. 60: 146-151.

Menaker, M., R. Roberts, J. Elliott, and H. Underwood. 1970. Extraretinal light perception in the sparrow, III. The eyes do not participate in photoperiodic photoreception. Proc. Nat. Acad. Sci. 67: 320-325.

Mikami, S. 1950. Niwatcri no shoka-tai jokjo ga karada seicho oyobi hoka no naibunpi-zoki ni oyobosu eikyo ni tsuite. (The effect of pinealectomy on body growth and on the other endocrine organs in the domestic fowl). Nippon juigaku Zasshi 12: 267-268.

Miller, R. A. 1961. Hypertrophic adrenals and their response to stress after lesions in the median eminence of totally hypophysectomized pigeons. Acta. Endocr. Copenhagen 37: 565-576.

Miller, R. A., and O. Riddle. 1942. The cytology of the adrenal cortex of normal pigeons and experimentally induced atrophy and hypertrophy. Am. J. Anat. 71: 311-335.

Miller, W.H., and M. L. Wolbarsht. 1962. Neural activity in the parietal eye of a lizard. Science 135: 316-317.

Mitchell, J. B. 1929. Experimental studies of the bird hypophysis. I. Effects of hypophysectomy in the Brown Leghorn fowl. Physiol. Zool. 2: 411-437.

Mitchell, M. E. 1970. Treatment of hypophysectomized hens with partially purified avian FSH. J. Reprod. Fertil. 22: 233-241.

Moore, R. Y. 1969. Visual pathways controlling neuroendocrine functions. In: Progress in Endocrinology. C. Gual, ed. Excerpta Medica Foundation, Amsterdam. pp. 490-494.

Morita, Y. 1966 a. Entladungsmuster pinealer Neurone der Regenbogenforelle (Salmo irideus) bei Belichtung des Zwischenhirns. Pflügers Archiv 289: 155-167.

Morita, Y. 1966 b. Absence of electrical activity of the pigeon's pineal organ in response to light. Experientia 22: 402.

Morita, Y., and E. Dodt. 1965. Nervous activity of the frog's epiphysis cerebri in relation to illumination. Experientia 21: 221-222.

Mrosovsky, N., and K. H. Tress. 1966. Plasticity of reactions to light in frogs and a possible role for the pineal eye. Nature, Lond. 210: 1174-1175.

Munns, T. W. 1970. Effects of different photoperiods on melatonin synthesis in the pineal gland of the canary (Serinus canarius) and testicular activity. Anat. Rec. 166: 352.

Nagra, C. L., J. G. Birnie, G. J. Baum, and R. K. Meyer. 1963. The role of the pituitary in regulating steroid secretion by the avian adrenal. Gen. Comp. Endocrinol. 3: 274-280.

Nalbandov, A. V., and L. E. Card. 1943. Effect of hypophysectomy of growing chicks. J. Exp. Zool. 94: 387-409.

Newcomer, W. S. 1959. Effects of hypophysectomy on some functional aspects of the adrenal gland of the chicken. Endocrinol. 65: 133-135.

Nikolaiczuk, N., and W. A. Maw. 1942. A preliminary study of the effect of sunlight, dubbing and fractionated anterior pituitary extract upon growth, endocrine glands, and sexual capacity of Single Comb White Leghorn cockerels. Poultry Sci. 21: 483-496.

Nir, I., U. Schmidt, N. Hirschmann, and F. G. Sulman. 1971. The effect of pinealectomy on rat plasma corticosterone levels under various conditions of light. Life Sci. 10: (I) 317-324.

Oishi, T. 1967. Effect of Photoperiod on the avian gonadal activity and photoreceptive system. Master's thesis. University of Kyoto, Kyoto, Japan.

Oishi, T., and M. Kato. 1968. Pineal organ as a possible photoreceptor in photoperiodic testicular response in Japanese quail. Memoirs of the Faculty of Science, Kyoto University, Series of Biology 2: 12-18.

Oishi, T., T. Konishi, and M. Kato. 1966. Investigation of photorecepting mechanism to control the gonadal development in Japanese quail. Environ. Cont. in Biol. 3: 87-90.

Oksche, A., and H. Kirschstein. 1969. Electronenmikroskopische Untersuchungen am Pinealorgan von Passer domesticus. Z. Zellforsch. 102: 214-241.

Oksche, A., and M. Vaupel-Von Harnack. 1966. Elektronenmikroskopische Untersuchungen zur Frage der Sinneszellen im Pinealorgan der Vögel. Z. Zellforsch. 69: 41-60.

Oksche, A., Y. Morita, and M. Vaupel-Von Harnack. 1969. Zur Feinstruktur and Funktion des Pinealorgans der Taube (Columba livia). Z. Zellforsch. 102: 1-30.

Ookawa, T. 1970 a. Effects of bilateral optic enucleation on body growth and gonad in young male chicks. Poultry Sci. 49: 333-334.

Ookawa, T. 1970 b. Some observations on behavior and reproductive organs in blinded chickens. Poultry Sci. 49: 1531-1534.

Opel, H. 1969. Transbuccal hypophysectomy in the Japanese quail. Poultry Sci. 48: 722-728.

Platt, C. S. 1953. Maintaining winter egg production by the use of dim red light. Poultry Sci. 32: 143-145.

Puntriano, G., and J. Meites. 1951. The effects of continuous light or darkness on thyroid function in mice. Endocrinol. 48: 217-224.

Quay, W. B. 1965. Retinal and pineal hydroxyindole-O-methyl transferase activity in vertebrates. Life Sci. 4: 983-991.

Quay, W. B. 1966. Rhythmic and light-induced changes in levels of pineal 5-hydroxyindoles in the pigeon (Columba livia). Gen. Comp. Endocrinol. 6: 371-377.

Quay, W. B. 1970. Endocrine effects of the mammalian pineal. Am. Zool. 10: 237-246.

Quay, W. B. and A. Renzoni. 1963. Studio comparativo e sperimentale sulla struttura e citologia della epifisi nei Passeriformes. (Comparative and experimental studies of pineal structure and cytology in Passeriform birds). Rivista di Biologia 56: 363-407.

Quay, W. B. and A. Renzoni. 1966. Osservazioni sulle "Cellule neurosecernenti commissuro-epifisarie" degli uccelli. (Studies on the "Commissuro-pineal neurosecretory cells" of birds). Rivista di Biologia 59: 231-266.

Quay, W. B., A. Renzoni, and R. M. Eakin. 1968. Pineal ultrastructure in Melopsittacus undulatus with particular regard to cell types and functions. Rivista di Biologia 61: 371-393.

Ralph, C. L. 1970. Structure and alleged functions of avian pineals. Am. Zool. 10: 217-235.

Ralph, C. L., and D. C. Dawson. 1968. Failure of the pineal body of two species of birds (Coturnix coturnix japonica and Passer domesticus) to show electrical responses to illumination. Experientia 24: 147-148.

Ralph, C. L., and K. B. Lane. 1969. Morphology of the pineal body of wild house sparrows (Passer domesticus) in relation to reproduction and age. Can. J. Zool. 47: 1205-1208.

Ralph, C. L., L. Hedlund, and W. A. Murphy. 1967. Diurnal cycles of melatonin in bird pineal bodies. Comp. Biochem. Physiol. 22: 591-599.

Reiter, R. J., R. A. Hoffman, and R. J. Hester. 1966. The effects of thiourea, photoperiod and the pineal gland on the thyroid, adrenal and reproductive organs of female hamsters. J. Exp. Zool. 162: 263-268.

Renzoni, A. 1967. La fisiologia dell'epifisi negli uccelli. I. Pinealectomia in Coturnix coturnix japonica. Soc. Ital. Biol. Speriment. Boll. 43: 585-588.

Renzoni, A. 1968. La fisiologia dell'epifisi negli uccelli. III. L'azione della melatonina sullo sviluppo sessuale di Coturnix coturnix japonica. Soc. Ital. Biol. Speriment. Boll. 44: 212-215.

Reynolds, E. S. 1963. The use of lead cytrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-213.

Ringoen, A. R. 1942. Effects of continuous green and red light illumination on gonadal response in the English sparrow, Passer domesticus (Linnaeus). Am. J. Anat. 71: 99-116.

Ringoen, A. R., and A. Kirschbaum. 1938. Factors responsible for the sexual cycle in the English sparrow, Passer domesticus (Linnaeus). Ocular stimulation and spermatogenesis; effect of increased light ration on ovarian development. J. Expt. Zool. 80: 173-191.

Roberts, J., and J. S. Carver. 1941. Electric light for egg production. Agr. Eng. 22: 357-360.

Rosner, J. M., G. Declercq de Pérez Bedés, and D. P. Cardinali. 1971. Direct effect of light on duck pineal explants. Life Sci. 10: (II) 1065-1069.

Rothchild, I. 1948. A simplified technique for hypophysectomy of the domestic fowl. Endocrinol. 43: 293-297.

Rowan, W. 1938. Light and seasonal reproduction in animals. Biol. Rev. 13: 374-402.

Rowe, J. W., J. R. Richert, D. C. Klein, and S. Reichlin. 1970. Relation of the pineal gland and environmental lighting to thyroid function in the rat. Neuroendocrinology 6: 247-254.

Sabatini, D. D., F. Miller and R. J. Barrnett. 1964. Aldehyde fixation for morphological and enzyme histochemical studies with the electron microscope. J. Histochem. and Cytochem. 12: 57-71.

Satodate, R., K. S. Hsieh, and M. Ota. 1970. Morphological changes in the pineal gland of the albino rat by hypophysectomy and ovariectomy. Experientia 26: 638-640.

Sayler, A., and A. Wolfson. 1967. Avian pineal gland: Progonadotropic response in the Japanese quail. Science 158: 1478-1479.

Sayler, A., and A. Wolfson. 1968 a. Influence of the pineal gland on gonadal maturation in the Japanese quail. Endocrinol. 83: 1237-1246.

Sayler, A., and A. Wolfson. 1968 b. Role of the eyes and superior cervical ganglia on the effects of light on the pineal and gonads of Japanese quail. Arch. Anat. Hist. Embryol. (Paris) 51: 615-626.

Sayler, A., and A. Wolfson. 1969. Hydroxyindole-O-methyl transferase (HIOMT) activity in the Japanese quail in relation to sexual maturation and light. Neuroendocrinology 5: 322-332.

Schapiro, S., and M. Salas. 1971. Effects of age, light, and sympathetic innervation on electrical activity of the rat pineal gland. Brain Res. 28: 47-55.

Schildmacher, H. 1963. Photoperiodischer Effekt und spektrales Helligkeitsempfinden bei einigen Vogelarten. Biol. Zent. 1: 31-44.

Scott, H. M., and L. F. Payne. 1937. Light in relation to the experimental modification of the breeding season of turkeys. Poultry Sci. 16: 90-96.

Shellabarger, C. J., and W. R. Breneman. 1950. The effects of pinealectomy on young White Leghorn cockerels. Indiana Acad. Sci. 59: 299-302.

Siegel, H. S. 1962. Critical ages for responses to ACTH in chicks. Gen. Comp. Endocrinol. 2: 385-388.

Singh, D. V., and C. W. Turner. 1967. Effect of melatonin upon thyroid hormone secretion rate and endocrine glands of chicks. Proc. Soc. Exp. Biol. Med. 125: 407-411.

Snyder, S. H. 1968. Development of enzyme activities and a circadian rhythm in pineal gland serotonin: Evidence for a non-retinal pathway of light to the pineal gland of new born rats. Adv. Pharmacol. 6: 301-305.

Soliman, F. A., H. M. Badawi, and Y. S. Ghanem. 1958. Influence of temperature and light on thyroid function. Nature 182: 57.

Stalsberg, H. 1965. Effects of extirpation of the epiphysis cerebri in 6-day chick embryos. Acta Endocrinologica (Copenhagen) Suppl. 97: 1-119.

Tanabe, Y., K. Himeno, and H. Nozaki. 1957. Thyroid and ovarian function in relation to molting in the hen. Endocrinol. 61: 661-666.

Taylor, A. N., and R. W. Wilson. 1970. Electrophysiological evidence for the action of light on the pineal gland in the rat. Experientia 26: 267-269.

Taylor, D. H., and D. E. Ferguson. 1970. Extraoptic celestial orientation in the southern cricket frog Acris gryllus. Science 168: 390-392.

Turner, K. B., and E. M. Benedict. 1932. Thyroid hyperplasia produced in chickens by ultraviolet light deficiency. J. Clin. Invest. 11: 761-774.

Ueck, M. 1970. Weitere Untersuchungen zur Feinstruktur und Innervation des Pinealorgans von Passer domesticus L. Z. Zellforsch. 106: 276-302.

Underwood, H., and M. Menaker. 1970 a. Photoperiodically significant photoreception in sparrows: Is the retina involved? Science 167: 298-301.

Underwood, H., and M. Menaker. 1970 b. Extraretinal light perception: Entrainment of the biological clock controlling lizard locomotor activity. Science 170: 190-193.

Van Brunt, E. E., M. D. Shepherd, J. R. Wall, W. F. Ganong, and M. T. Clegg. 1964. Penetration of light into the brain of mammals. Ann. New York Acad. Sci. 117: 217-227.

Viggiani, E., M. Ciesla, and O. L. Russo. 1970. Penetration of light through the skull and the brain of the rat at various ages. Measurements taken separately for the skin and the bone. Boll. Soc. Ital. Biol. Sper. 46: 470-473.

Wetterberg, L., E. Geller, and A. Yuwiler. 1970 a. Harderian gland: An extraretinal photoreceptor influencing the pineal gland in neonatal rats? Science 167: 884-885.

Wetterberg, L., A. Yuwiler, R. Ulrich, E. Geller, and R. Wallace. 1970 b. Harderian gland: Influence on pineal hydroxyindole-O-methyltransferase activity in neonatal rats. Science 170: 194-196.

Williams, B. J. 1969. Light intensity and sexual maturity. Am. J. Physical. Anthropology 30: 151-152.

Williams, C. M., P. L. Adkisson, and C. Walcott. 1965. Physiology of insect diapause. XV. The transmission of photoperiod signals to the brain of the oak silkworm, Antheraea pernyi. Biol. Bull. 128: 497-507.

Wilson, W. O., A. E. Woodard, and H. Abplanalp. 1956. The effect and after-effect of varied exposure to light on chicken development. Biol. Bull. 111: 415-422.

Winget, C. M., C. A. Warren, and C. W. DeRoshia. 1967. Interrelationships of the pineal gland, the diencephalon and the pituitary (Gallus domesticus). Am. Zool. 7: 732.

Wolfson, A. 1965. Circadian rhythm and the photoperiodic regulation of the annual reproductive cycle in birds. In: Circadian Clocks. J. Aschoff ed., North-Holland Publishing Co., Amsterdam, pp. 370-378.

Woodard, A. E., J. A. Moore, and W. O. Wilson. 1968. Effect of wavelength of light on growth and reproduction in Japanese quail (Coturnix coturnix japonica). Poultry Sci. 47: 1733-1734.

Wurtman, R. J. 1967. Effects of light and visual stimuli on endocrine function. In: Neuroendocrinology II. L. Martini and W. G. Ganong, ed., Academic Press, New York and London. pp. 19-59.

Wurtman, R. J., and F. Anton-Tay. 1969. The mammalian pineal as a neuroendocrine transducer. Rec. Prog. Horm. Res. 25: 493-514.

Wurtman, R. J., and J. Weisel. 1969. Environmental lighting and neuroendocrine function: Relationship between spectrum of light source and gonadal growth. Endocrinol. 85: 1218-1221.

Wurtman, R. J., J. Axelrod, and J.E. Fischer. 1964. Melatonin synthesis in the pineal gland: Effect of light mediated by the sympathetic nervous system. Science 143: 1328-1330.

Wurtman, R. J., J. Axelrod, and D. E. Kelly. 1968. The pineal. Academic Press, New York and London.

Yoshida, M., and N. Millott. 1959. Light sensitive nerve in an Echinoid. Experientia 15: 13-14.

Young, J. Z. 1935. The photoreceptors of lampreys. II. The functions of the pineal complex. J. Exp. Biol. 12: 254-270.

Zadula, J., J. Roszkowski, and S. Cakala. 1969. Effect of pinealectomy on the weight and histological changes in the testes and adrenals of cockerels. Acta Physiol. Pol. 20: 95-101.

Zweig, M., S. H. Snyder, and J. Axelrod. 1966. Evidence for a nonretinal pathway of light to the pineal gland of newborn rats. Proc. Nat. Acad. Sci. 56: 515-520.

Figure 25. Electron micrograph showing discs in the outer segment of a pineal photoreceptor cell. Numerous mitochondria can be seen in the inner segment. Newly hatched quail.

: disc, OS: outer segment, IS: inner segment, MT: mitochondria, CI: cilium, GA: Golgi apparatus, L: lumen

x 30,400

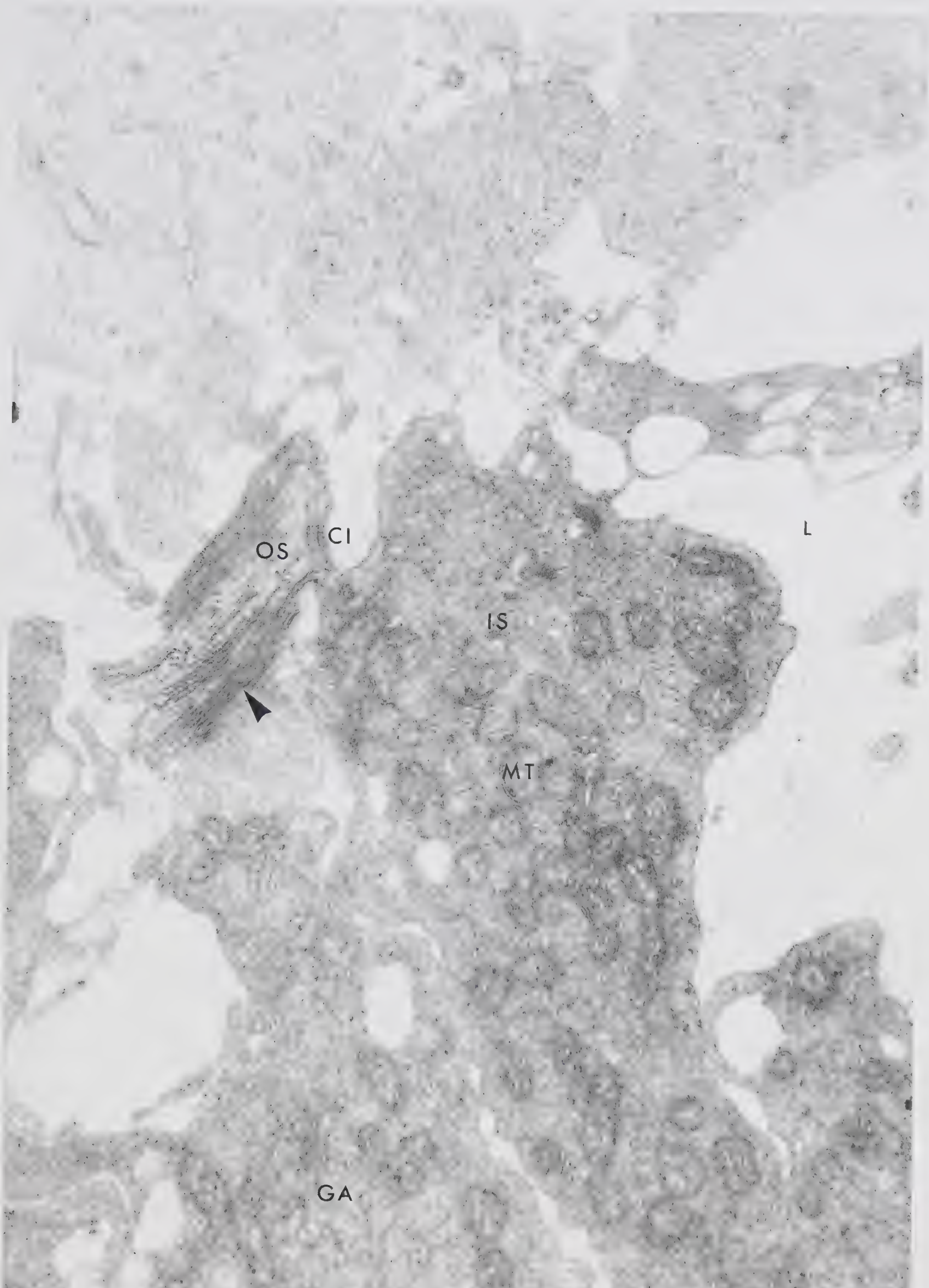

OS
CI
L
IS
MT
GA

Figure 26. A photoreceptor cell process with prominent Golgi apparatus, in the lumen of a pineal lobule. Membrane-limited dense-cored vesicles (1,000 - 1,300 Å in diameter: ▲) and numerous vesicles without a dense-core (400 - 800 Å in diameter: ↑) can be seen. Newly hatched quail.

GA: Golgi apparatus, MT: mitochondria,
ER: endoplasmic reticulum, L: lumen
x 52,000

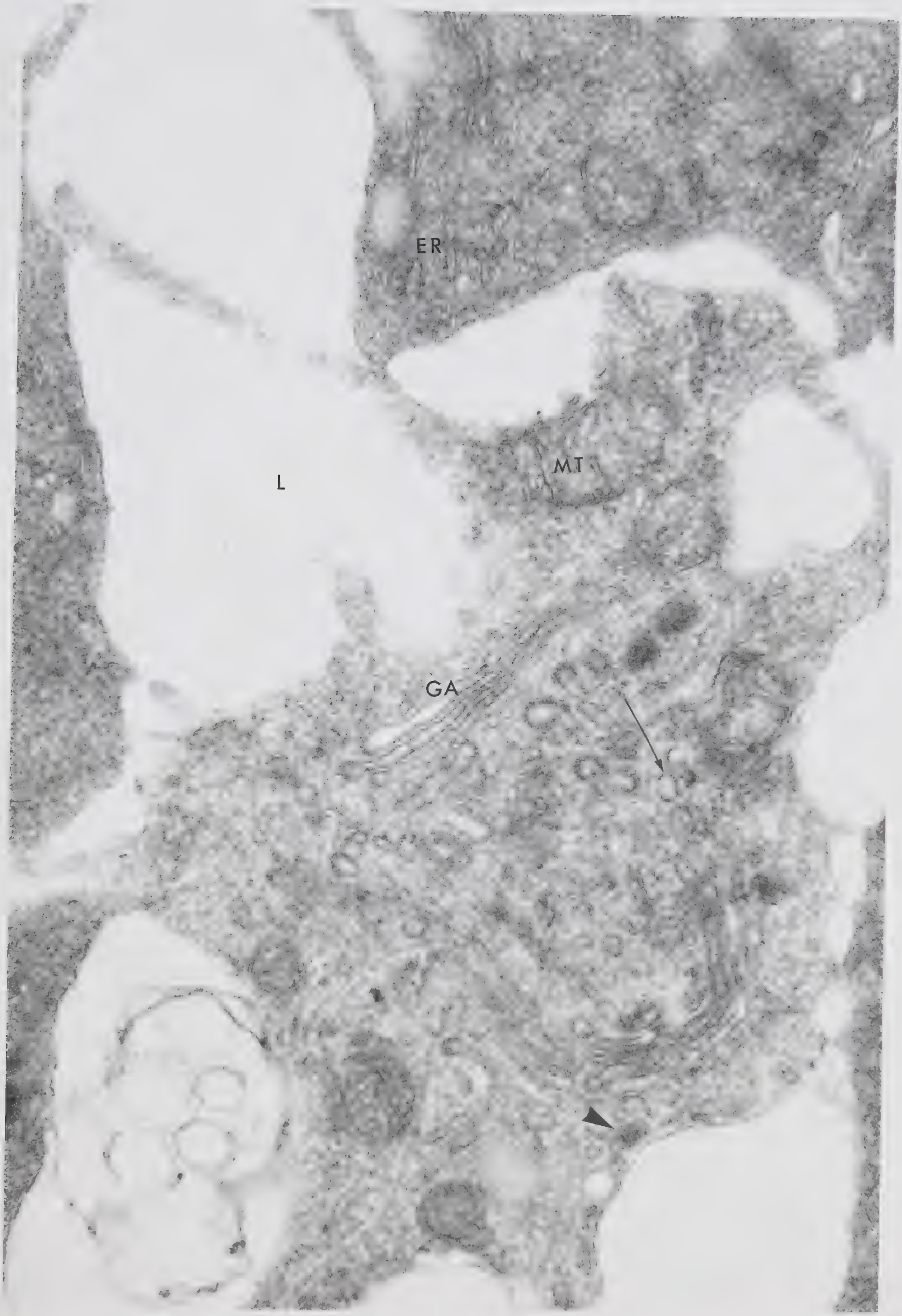

ER
MT
L
GA

Figure 27. Apical portions of a photoreceptor cell (dark) and a supportive cell (light). A cell junction (ZA) is observed between the neighbouring cells. At this region, the photoreceptor cell cytoplasm is constricted into a neck which separates the inner segment from the remainder of the cell. Both cell types have many mitochondria in the inner segment. At the neck, numerous microtubules run parallel to the long axis of the cell. Cilia of the (9 + 0) configuration can be seen in the lumen. Newly hatched quail. IS: inner segment, ZA: zonula adherens, CI: cilium, MT: mitochondria, L: lumen, PC: photoreceptor cell, SC: supportive cell x 18,400

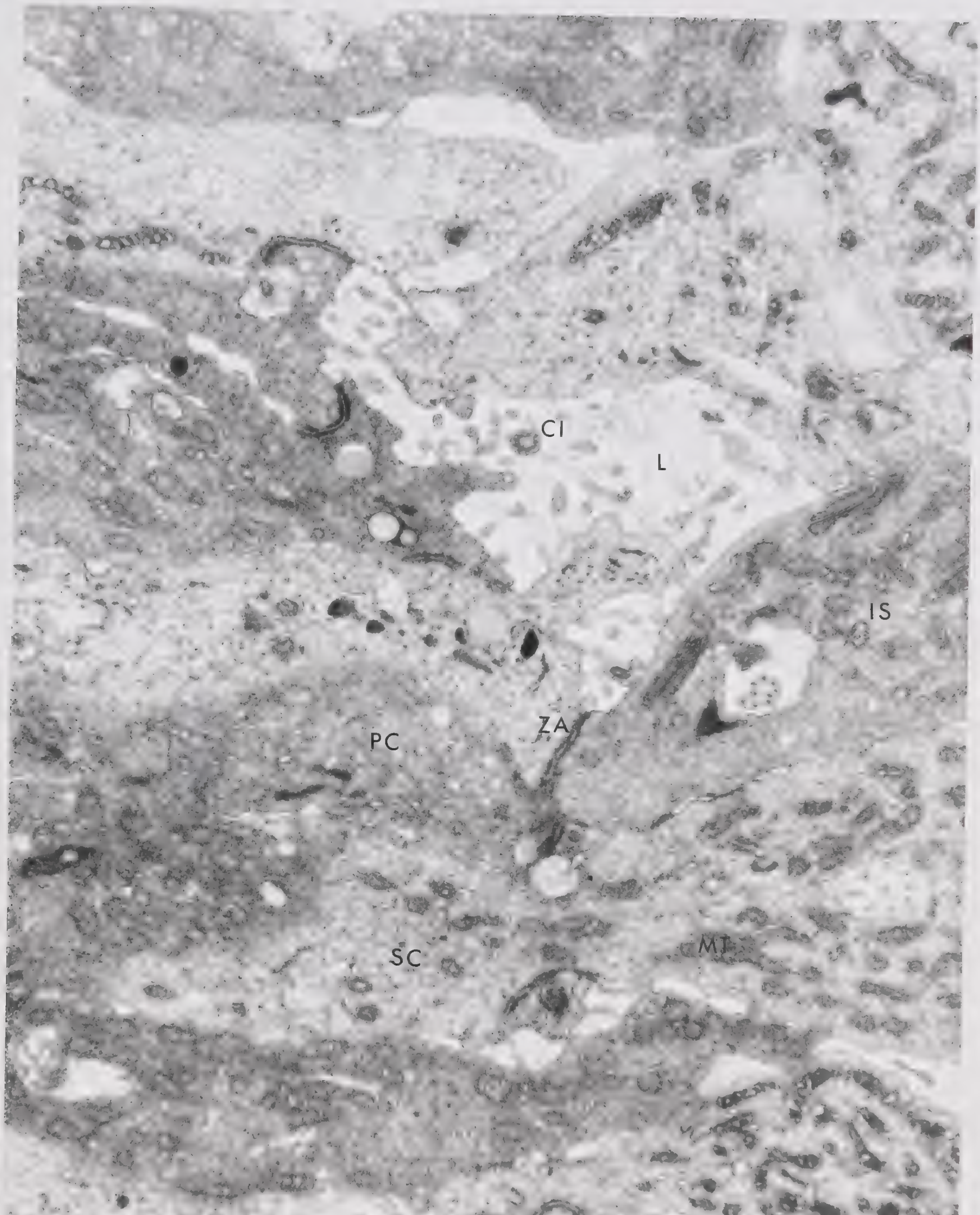

CI
L
IS
ZA
PC
SC
MT

Figure 28. A large nucleus with prominent nucleolei and slim cytoplasm, characterizing the dark photoreceptor cell. Mitochondria and endoplasmic reticulum are richer in the light-staining supportive cell than in the photoreceptor cell. Newly hatched quail.

N: nucleus, NO: nucleolus, GA: Golgi apparatus, PC: photoreceptor cell, SC: supportive cell

x 18,400

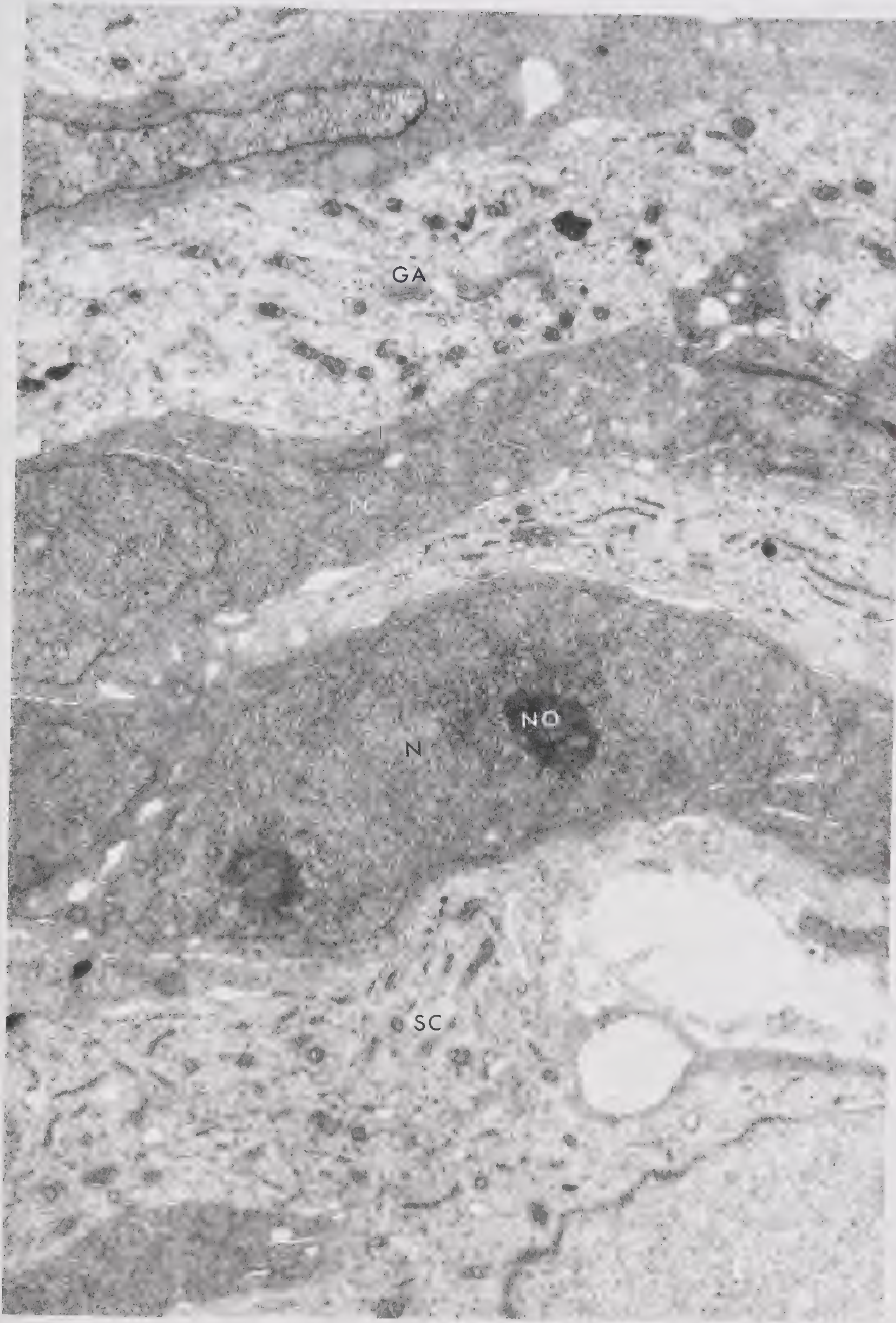

GA
N
NO
N
SC

Figure 29. Basal portions of several photoreceptor cells. Many cell processes protrude toward the pericapillary area. These processes are intermingled with nerve fibers. In the nerve ending, synaptic vesicles (about 500 Å in diameter - ↑) and membrane-limited dense-cored vesicles (about 1,000 Å in diameter - ▲) can be seen. Newly hatched quail.

x 18,400

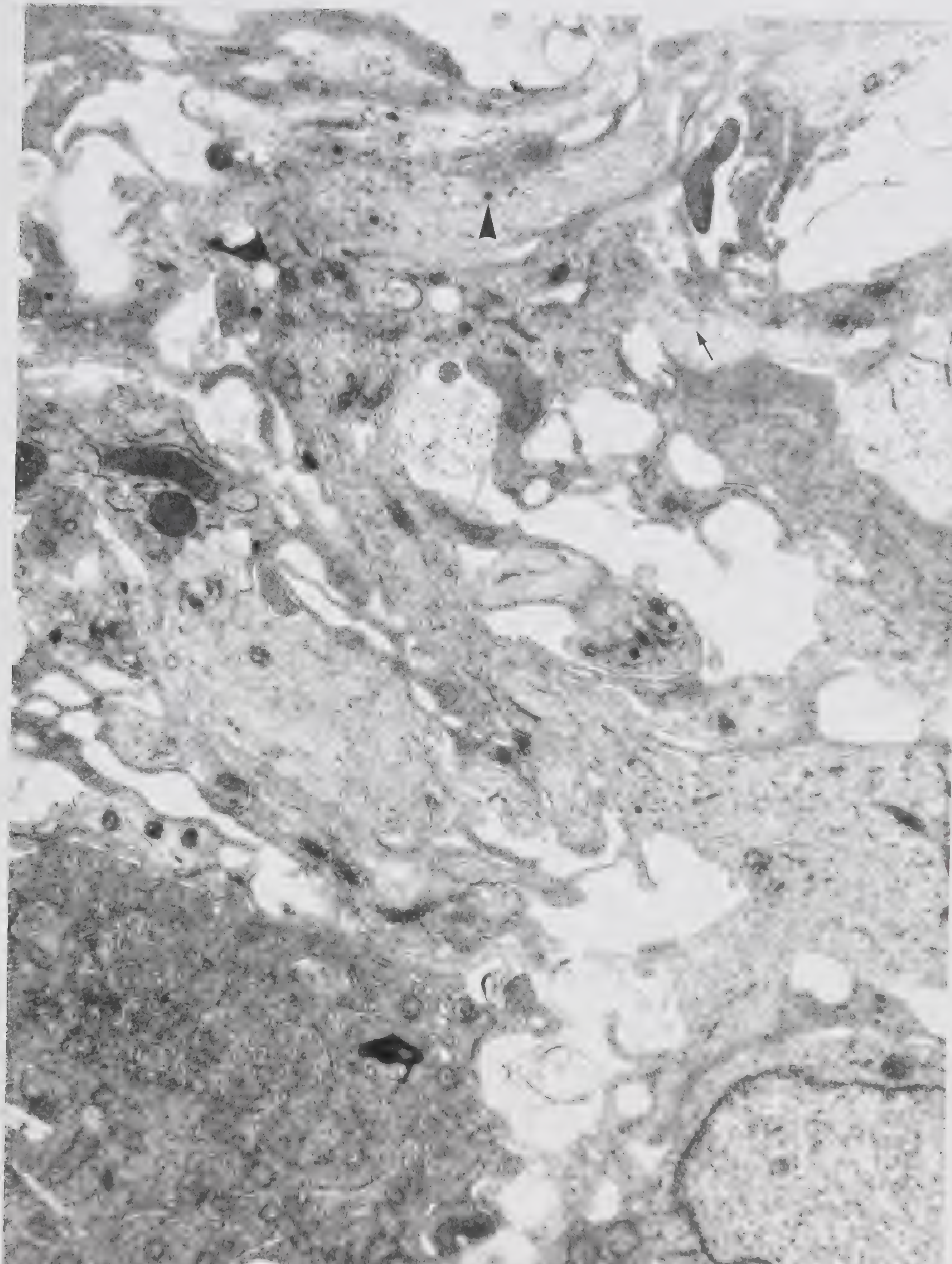


Figure 30. A ganglion cell body with numerous mitochondria, a nerve ending and the processes of a photoreceptor cell can be seen in the pericapillary area. The photoreceptor cell processes contain membrane-limited dense-cored vesicles (800 - 1,200 Å in diameter - ↑).
A nerve ending shows synaptic vesicles and membrane-limited dense-cored vesicles (▲).
Newly hatched quail.
BM: basement membrane; GC: ganglion cell;
NE: nerve ending
x 30,400

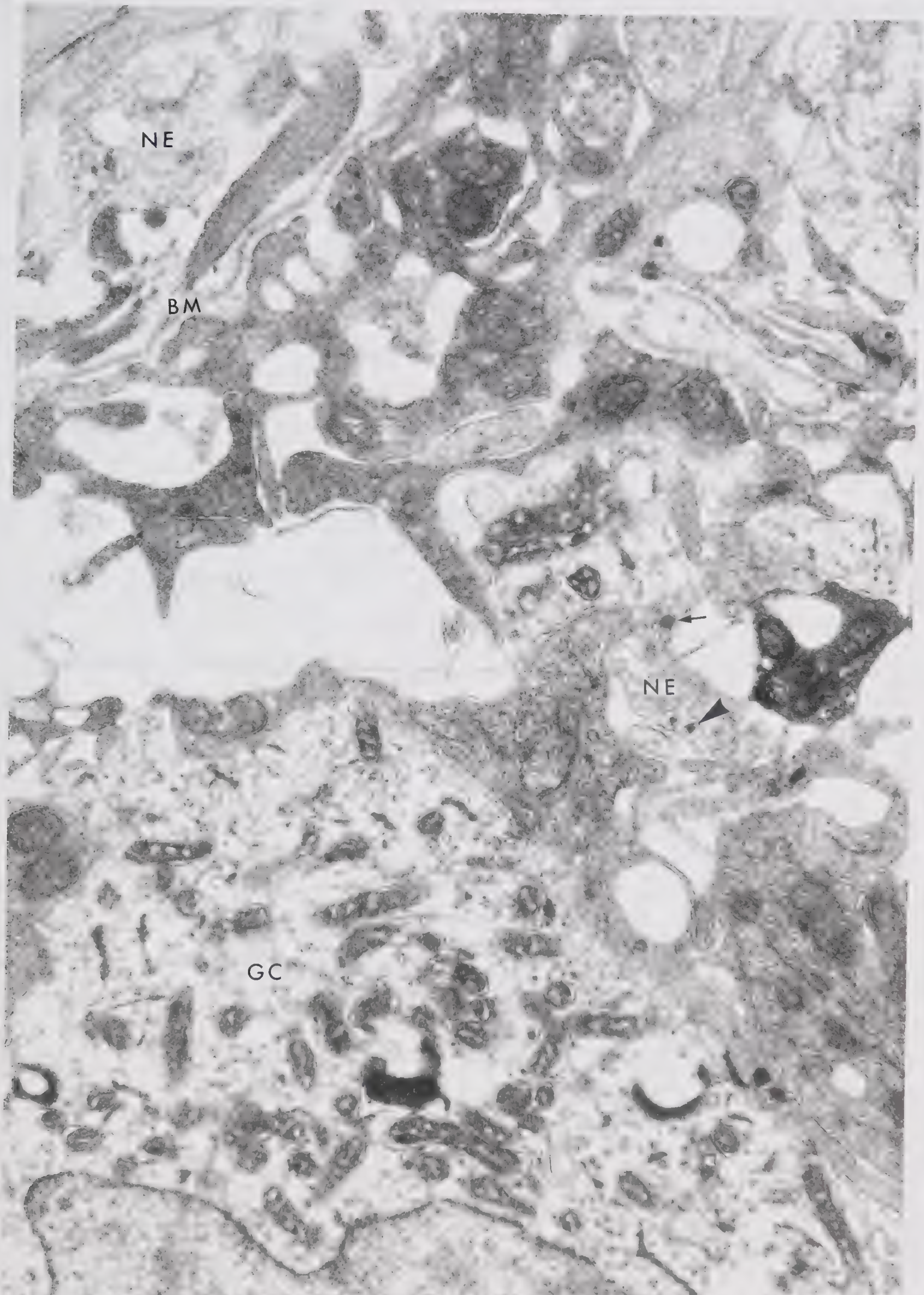

NE
BM
NE
GC

Figure 31. A nerve ending adjacent to several photoreceptor cell processes. The processes of the photoreceptor cell contains membrane-limited secretory granules (↑), and have many vacuoles (VA). In the nerve ending, there are prominent mitochondria and synaptic vesicles. Membrane-limited dense-cored vesicles (▲) of the same size as those of the photoreceptor cell, can also be seen in the nerve ending. Newly hatched quail.

NE: nerve ending, PP: process of photoreceptor cell, VA: vacuoles, BM: basement membrane

x 52,000

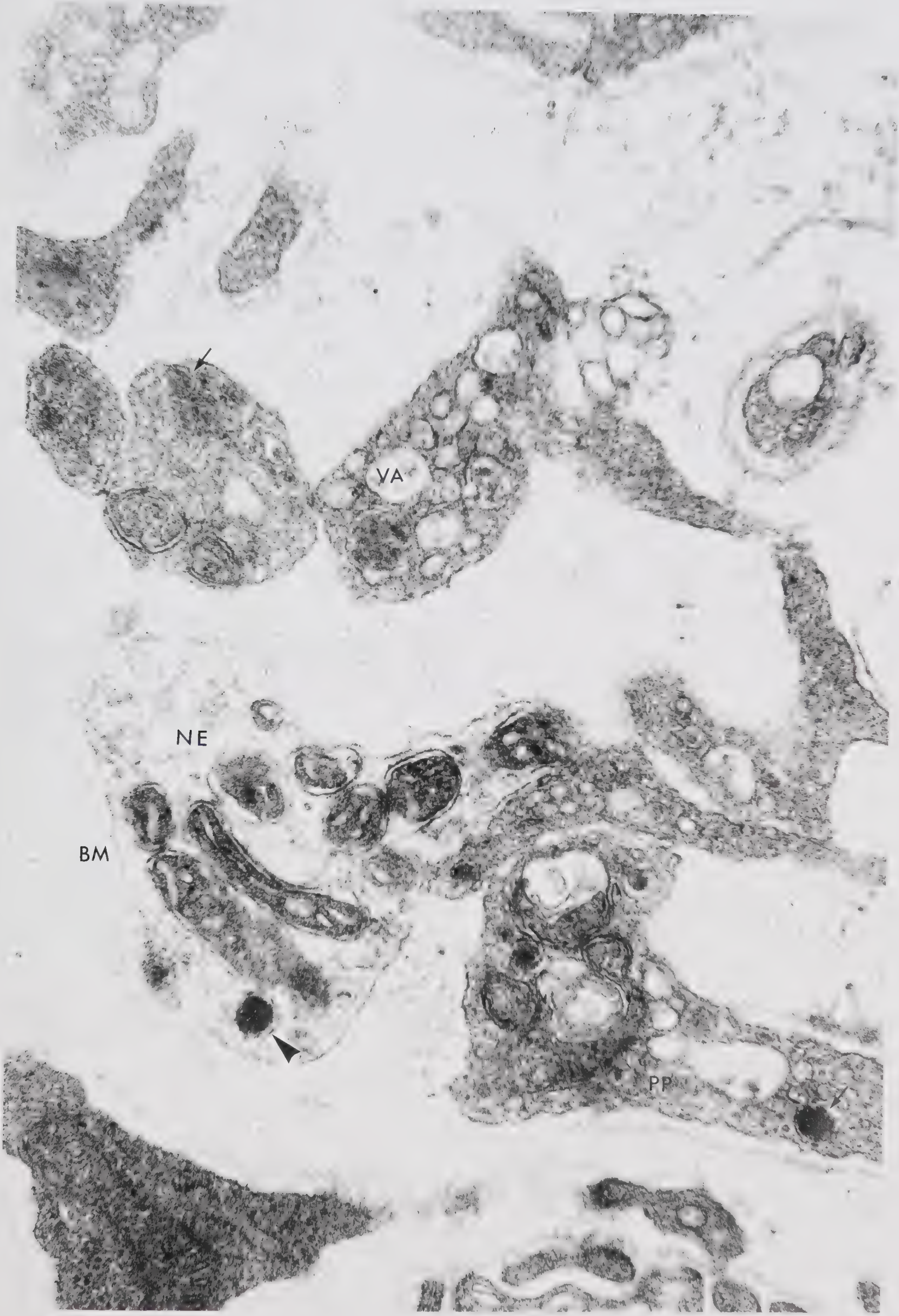
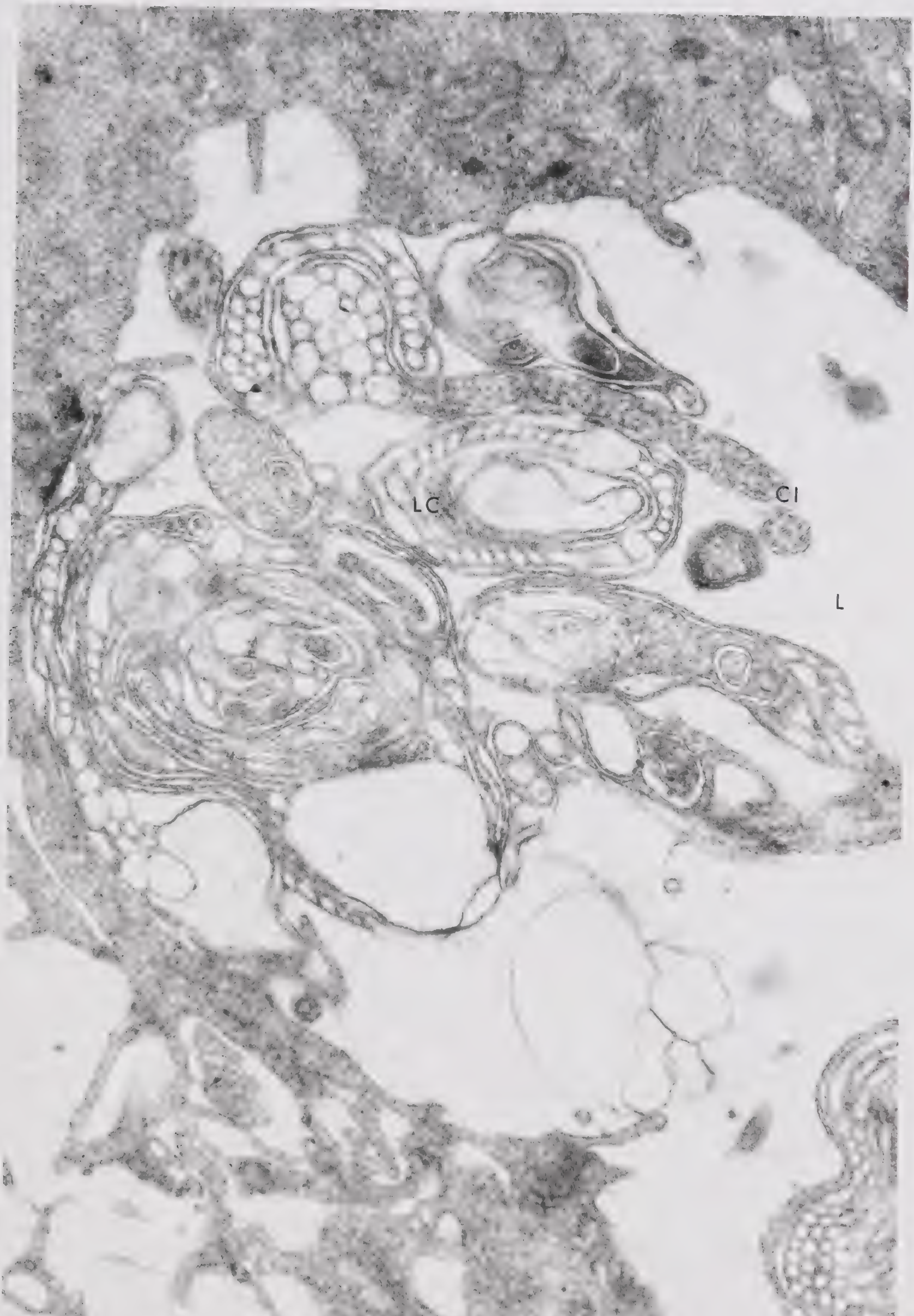

VA
NE
BM
PP

Figure 32. The lamellar complex of a photoreceptor cell. Note the cilium connected to the lamellar complex. Adult quail.

L: lumen, Ci: cilium (9 + 2) type, LC: lamellar complex

x 30,400



LC
CI
L

Figure 33. A cilium connected to the inner segment of a photoreceptor cell. Adult quail.

CI: cilium, ZA: zonula adherens, IS: inner segment, MV: microvilli

x 30,400

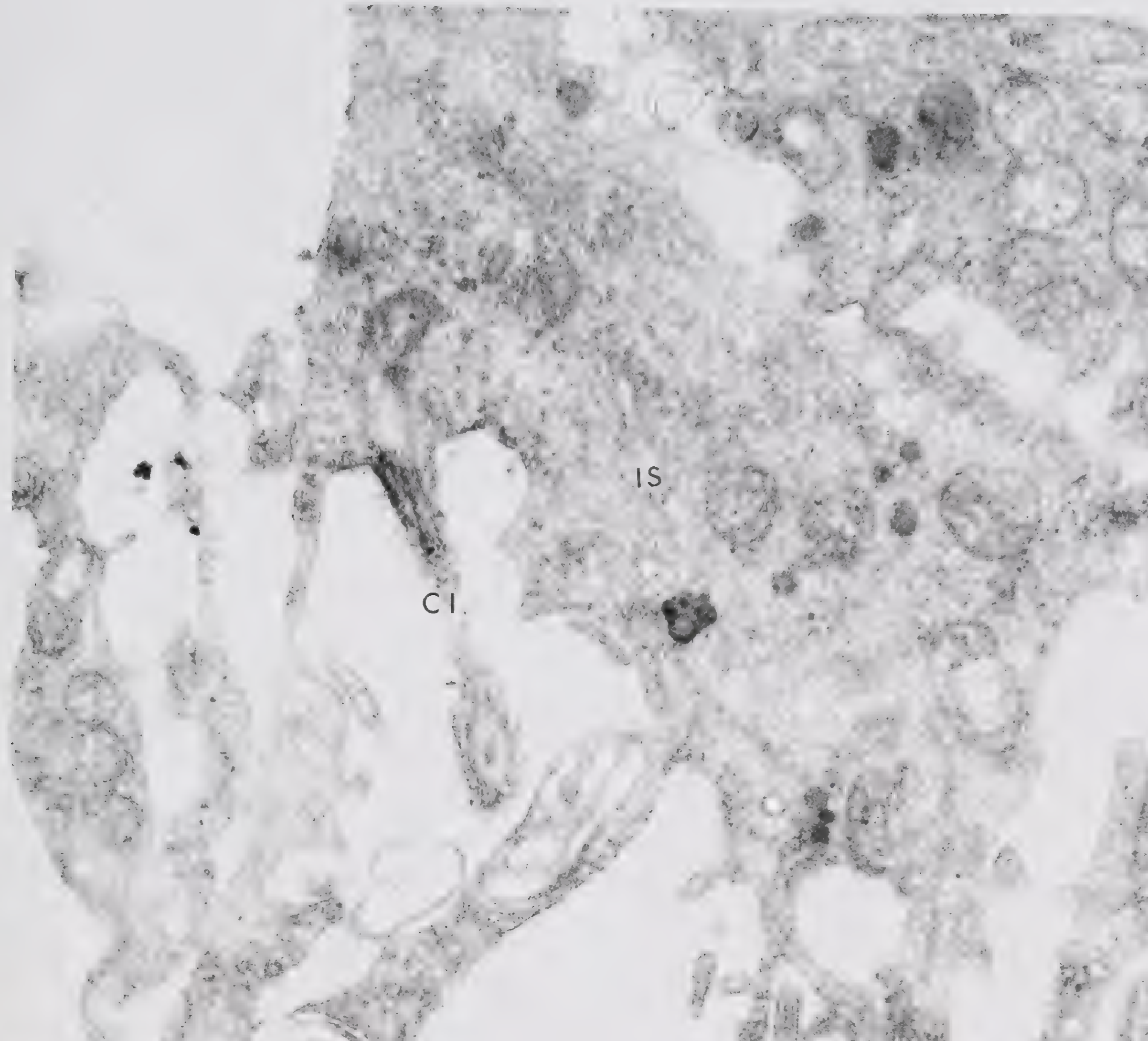

IS
CI
MV
ZA

Figure 34. The apical portion of a photoreceptor cell and a supportive cell. Both cells contain numerous mitochondria. Adult quail.

L: lumen, PC: photoreceptor cell, SC: supportive cell, CI: cilium, MV: microvilli, ZA: zonula adherens, IS: inner segment

x 18,400

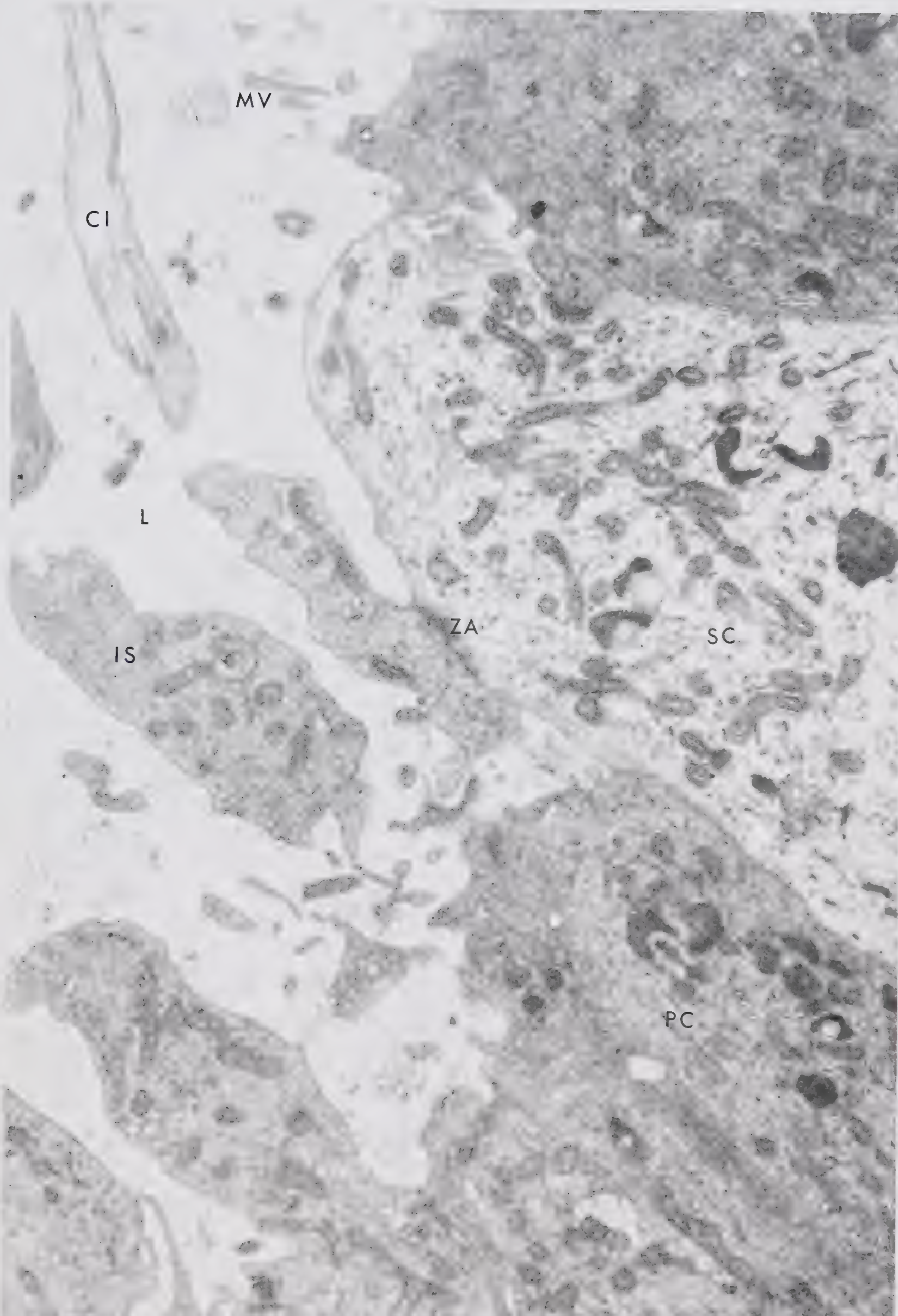

MV
CI
L
IS
ZA
SC
PC

Figure 35. A portion of the cytoplasm of a photoreceptor cell, containing abundant Golgi apparatus, with vesicles. Endoplasmic reticulum and scattered ribosomes can also be seen. Adult quail.

N: nucleus, MT: mitochondria, GA: Golgi apparatus, ER: endoplasmic reticulum,

↑: vesicles

x 52,000

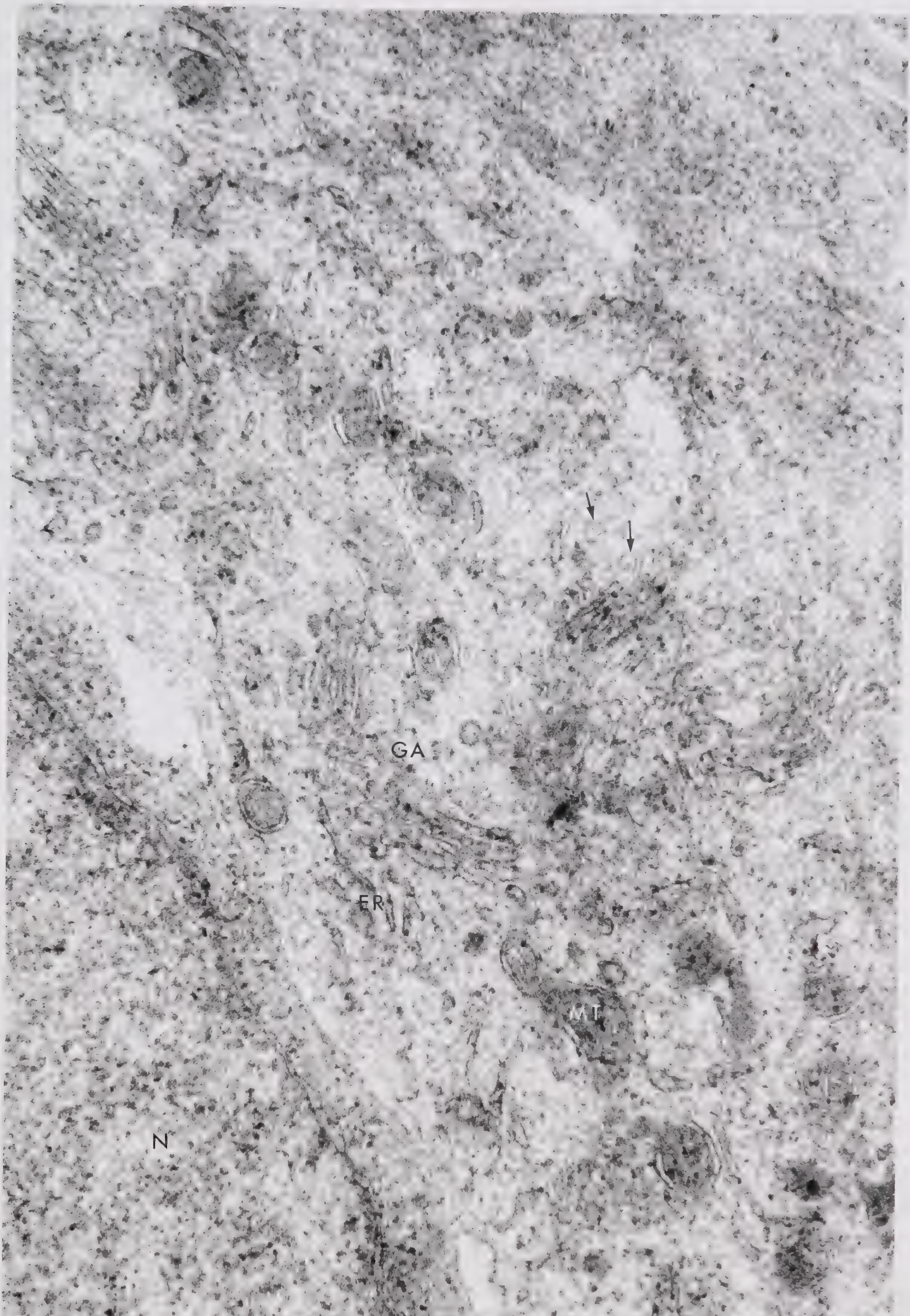

GA
ER
MT
N

Figure 36. A ganglion cell body embedded in the processes of several photoreceptor cells and surrounded by a basement membrane. The extended ganglion cell processes (probably axon and dendrite) contain numerous filaments (↑). The peri-nuclear cytoplasm is rich in mitochondria and endoplasmic reticulum. Adult quail.

GC: ganglion cell, PP: process of photo-receptor cell, BS: basement membrane

x 11,200

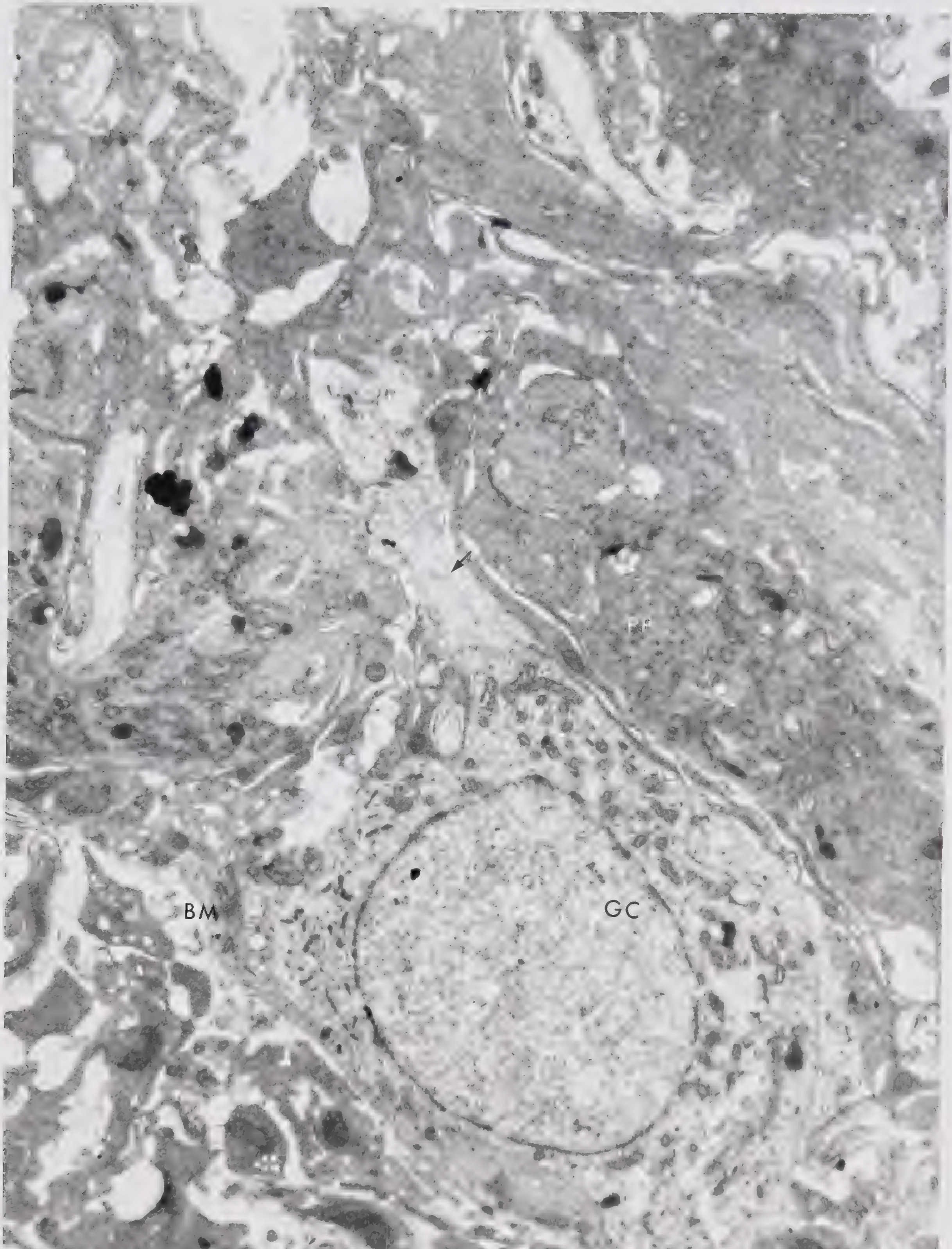

BM
GC

Figure 37. Myelinated (MN) and unmyelinated (UN) nerve fibers in the pericapillary area. Prominent Schwann cells surround several of the nerve fibers. Adult quail.

x 30,400

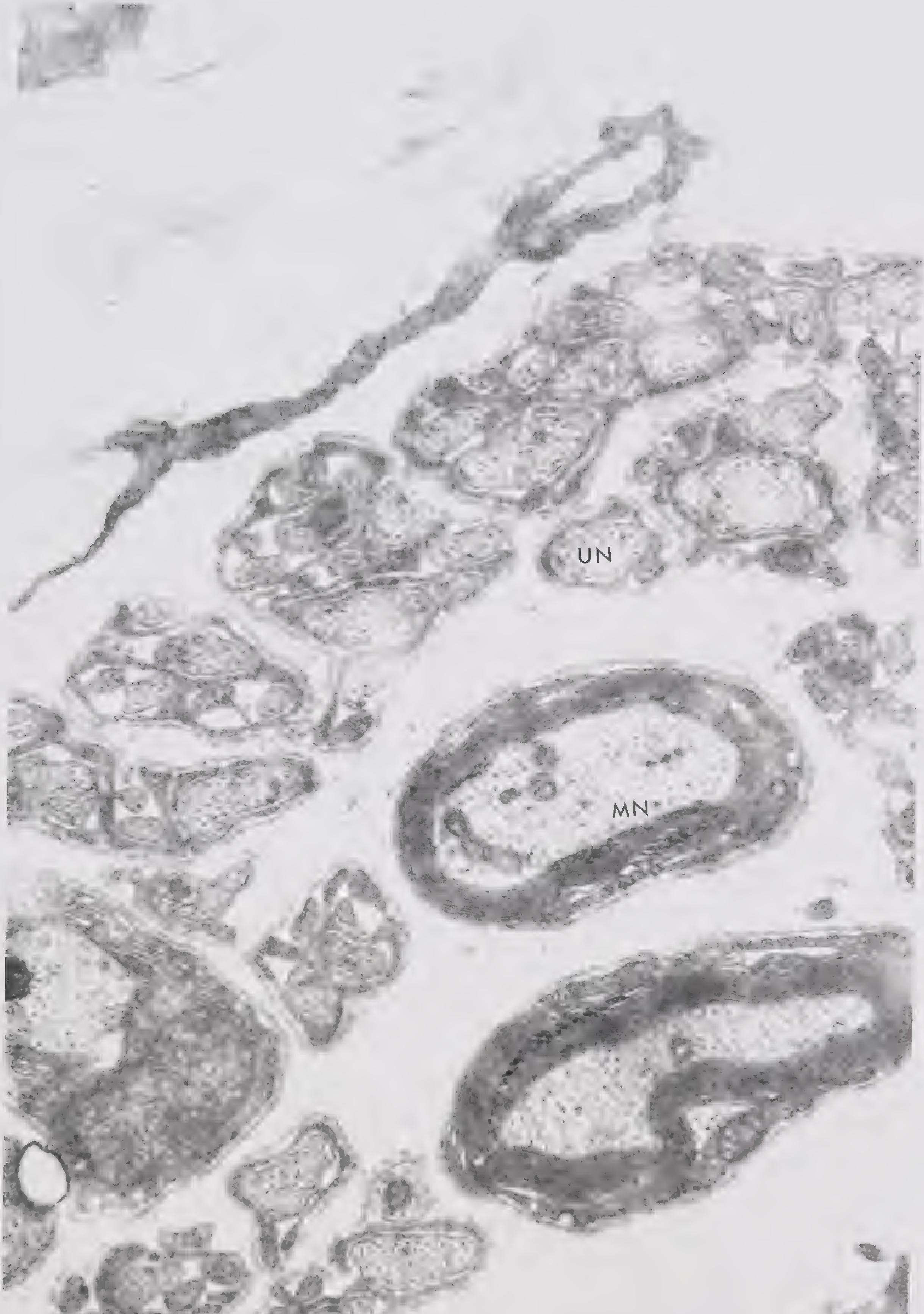

UN
MN

Figure 38. Numerous membrane-limited dense-cored vesicles (secretory granules) (800 - 1,200 Å in diameter - ↑) in the processes of a photoreceptor cell, close to the basement membrane (BM). Adult quail, maintained under continuous light. x 30,400

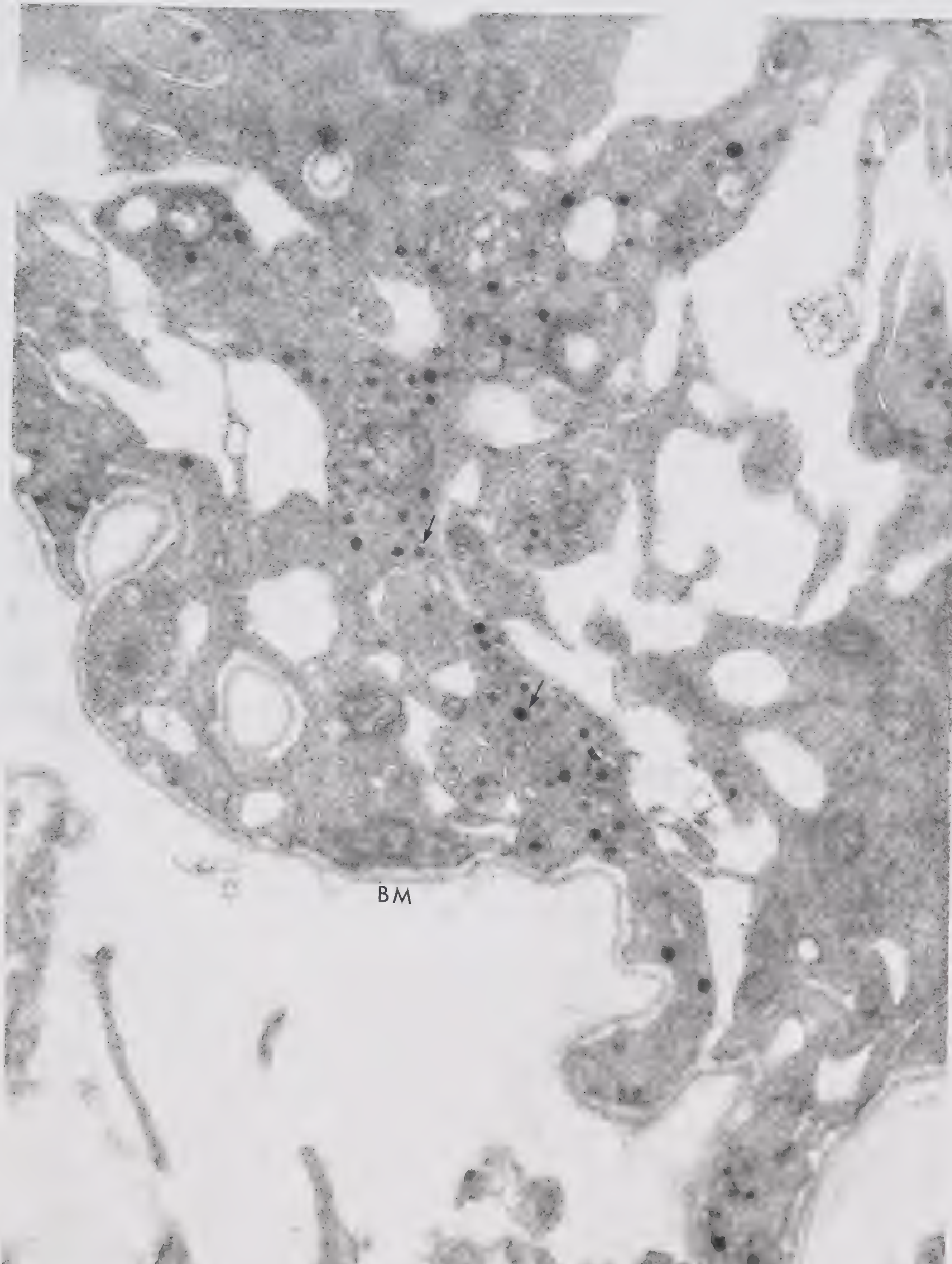

BM

Figure 39. Secretory granules of a photoreceptor cell in higher magnification than Figure 38. Adult quail maintained under continuous light. x 89,600

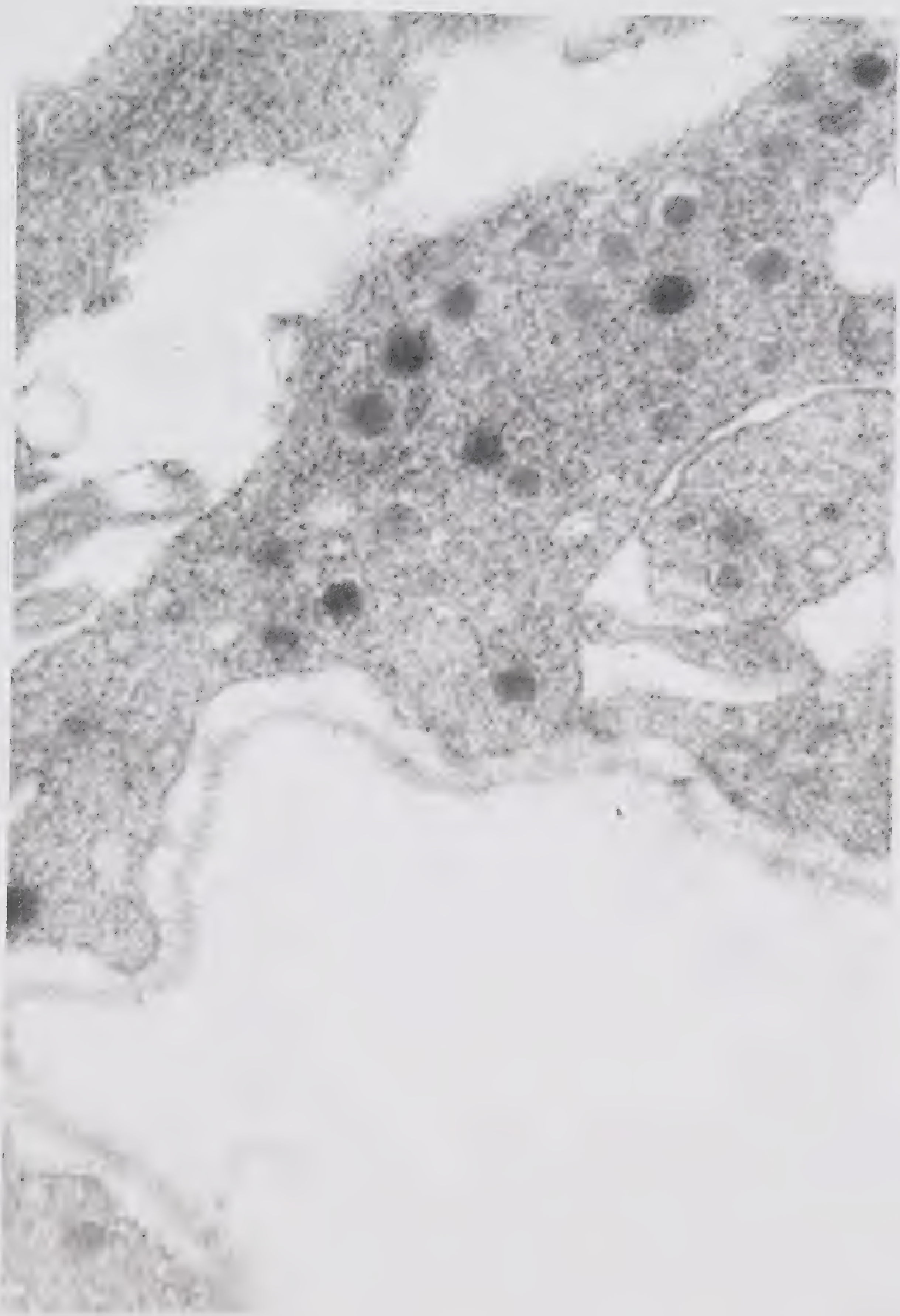


Figure 40. Processes of a photoreceptor cell. Secretory granules (▲) and a vesicle-crowned synaptic ribbon (↑) can be seen. Adult quail maintained under continuous light.

x 52,000

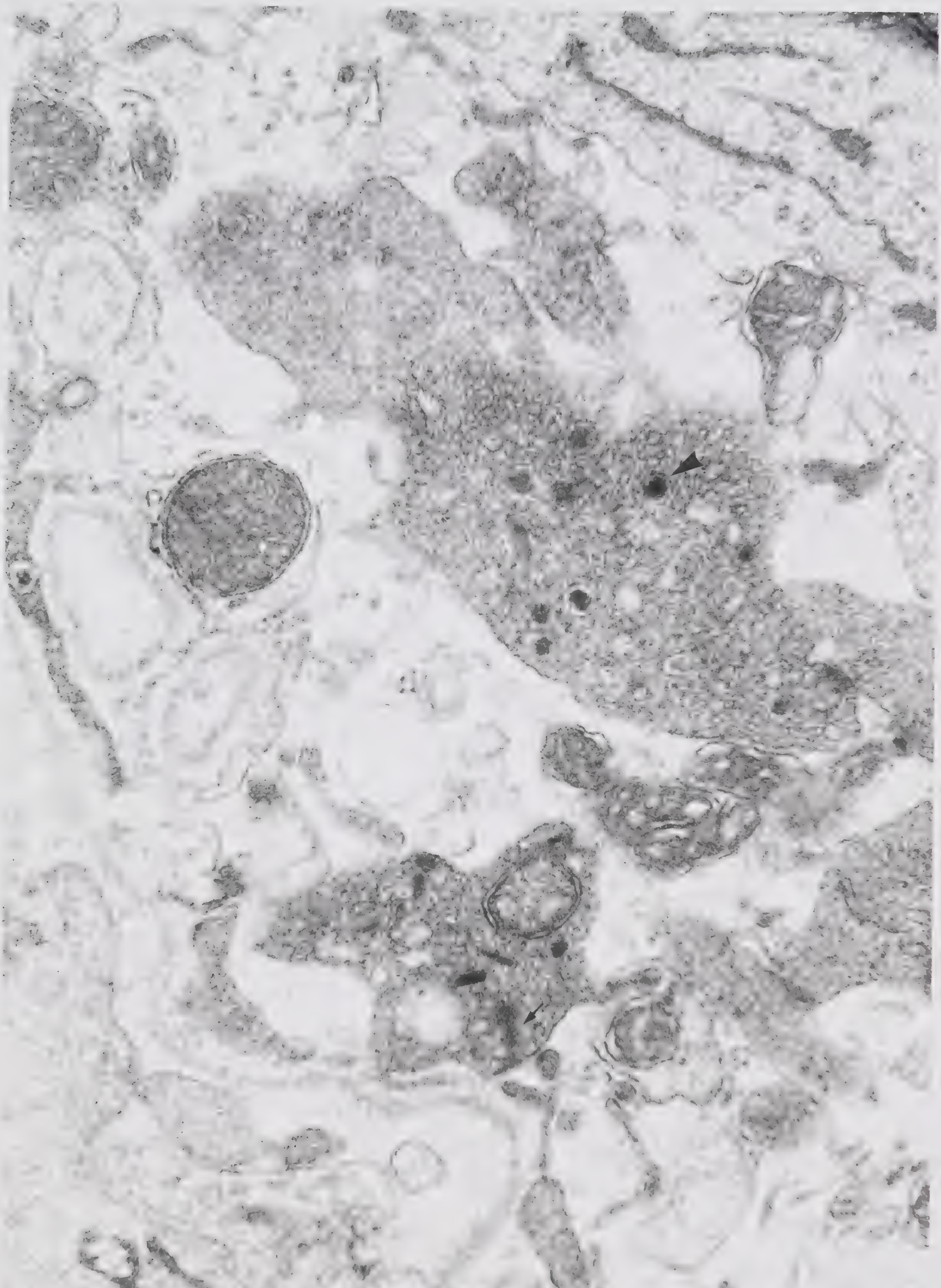


Figure 41. Processes of a photoreceptor cell (PP) and nerve endings (NE) in the pericapillary area. Half depleted secretory granules (↑) can be seen in the nerve ending. Adult quail maintained under continuous light.

CF: collagen fibril

x 52,000

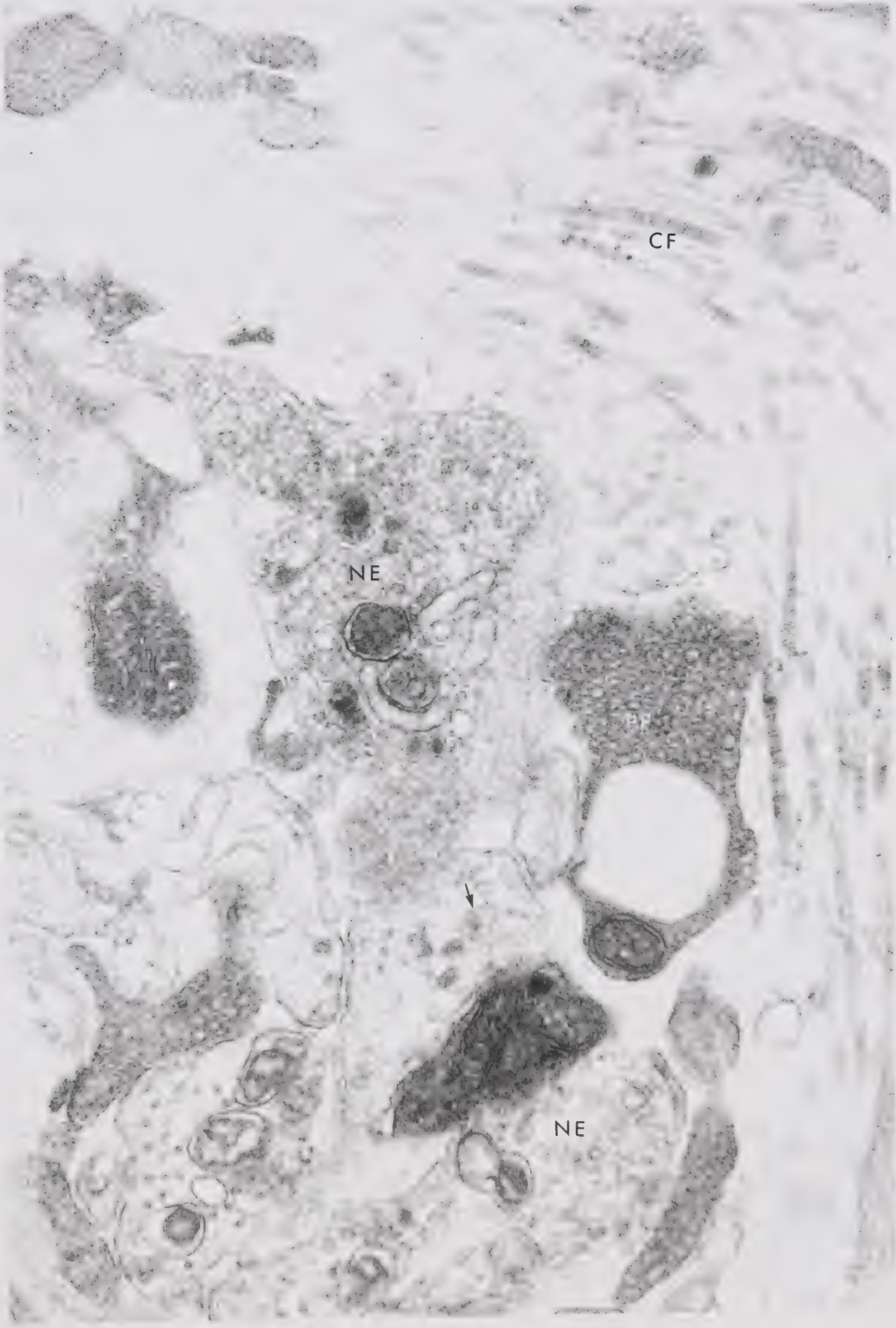

CF
NE
NE

Figure 42. A nerve ending (NE) surrounded by photoreceptor cell processes (PP). Both cells contain secretory granules (↑) and synaptic vesicles (▲). Desmosome like junction can be seen between the two cell types (▲). Adult quail maintained under continuous light.

CF: collagen fibril

x 52,000

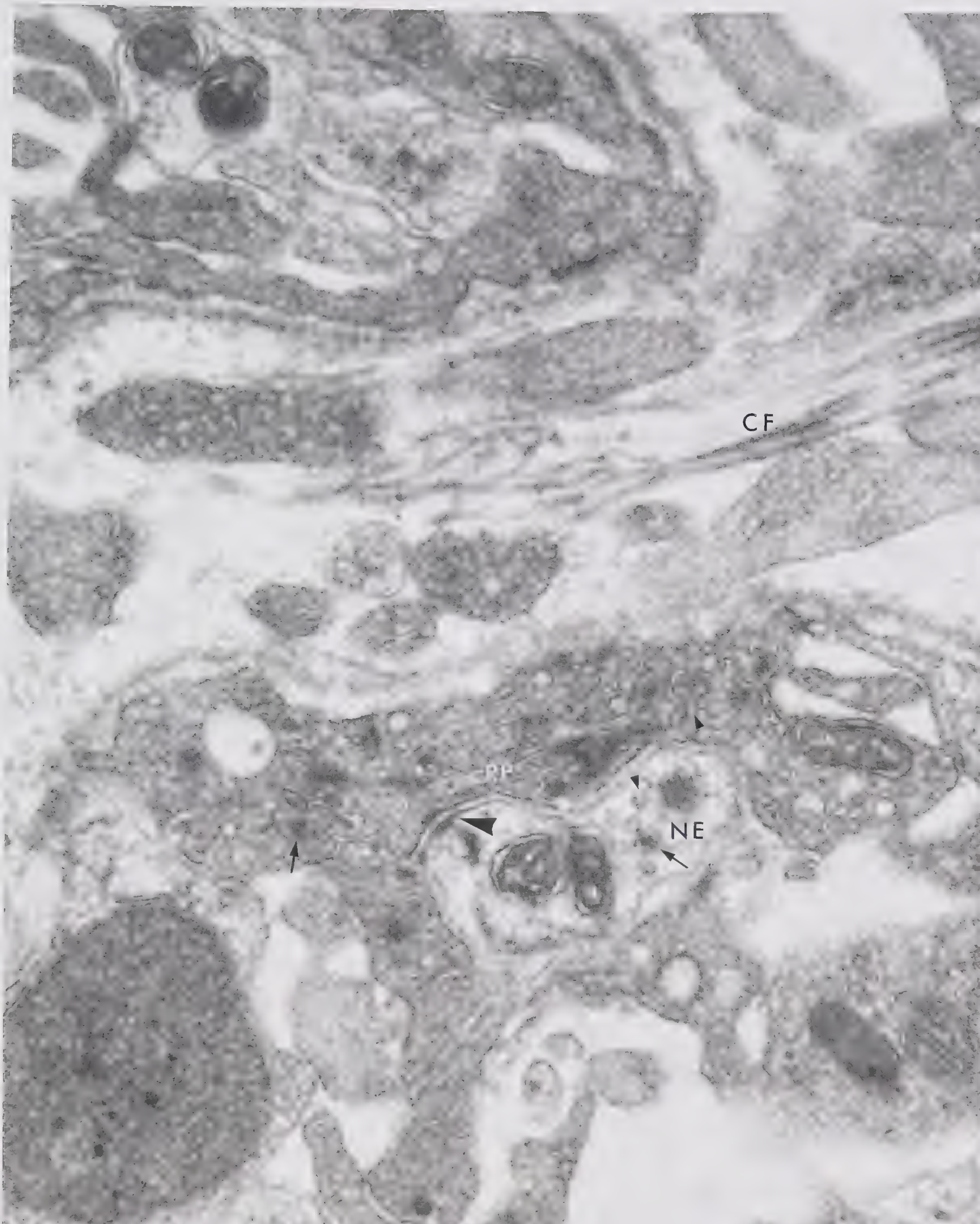

CF
RP
NE

Figure 43. Photoreceptor cell processes bordering the lumen of a pineal lobule. Membrane-limited dense-cored vesicles (1,000 - 1,300 Å in diameter - ↑) can be seen close to the lumen. A lamellar complex (LC) lies in the lumen. Adult quail maintained in continuous darkness for 1 week.

x 30,400

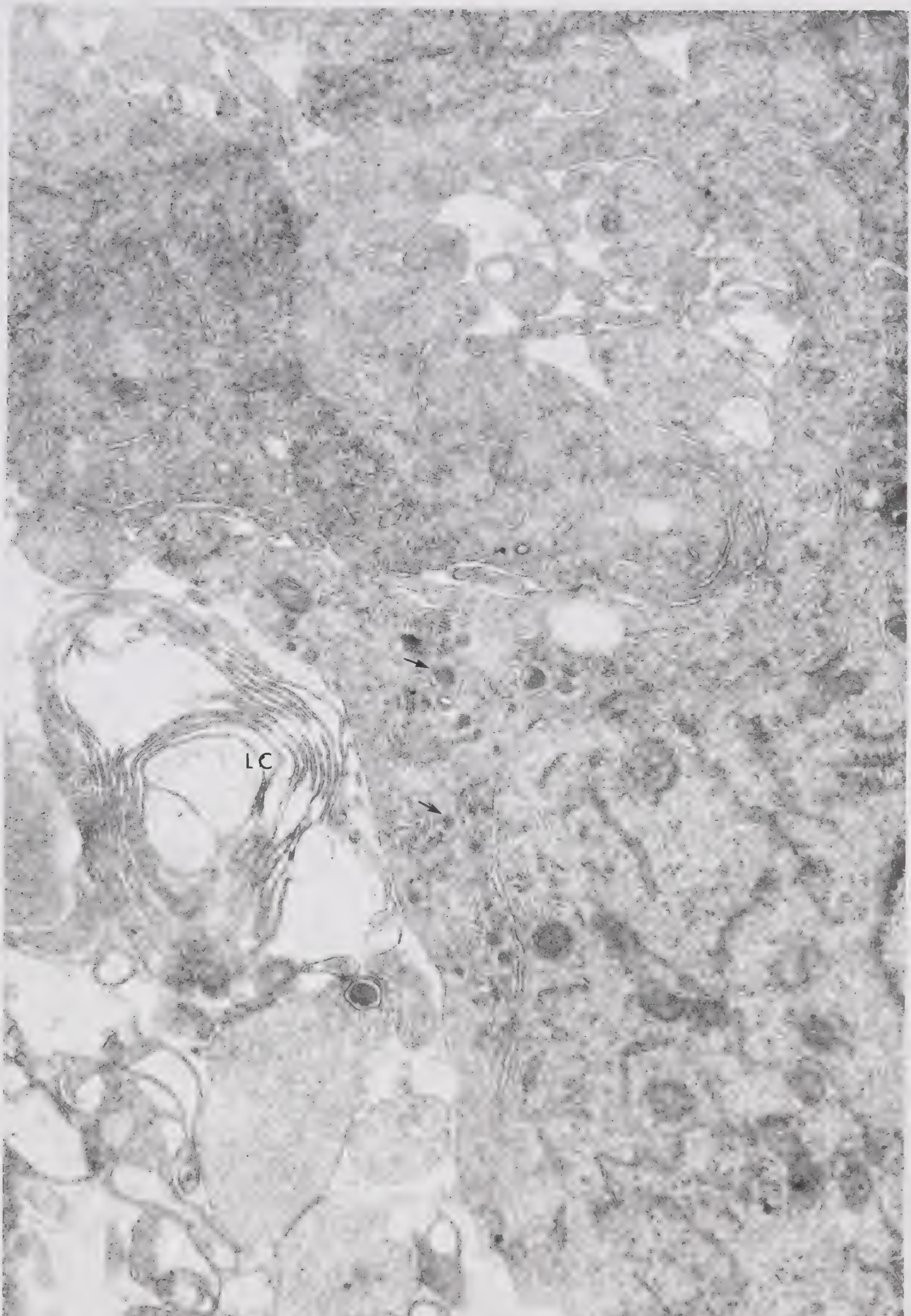

LC

Figure 44. The process of a photoreceptor cell, projecting into the lumen of a lobule, contains membrane-limited dense-cored vesicles (↑). The neighboring supportive cell (SC) shows Golgi apparatus (GA), endoplasmic reticulum (ER) and scattered ribosomes. Adult quail maintained under continuous darkness for 1 week.

x 30,400

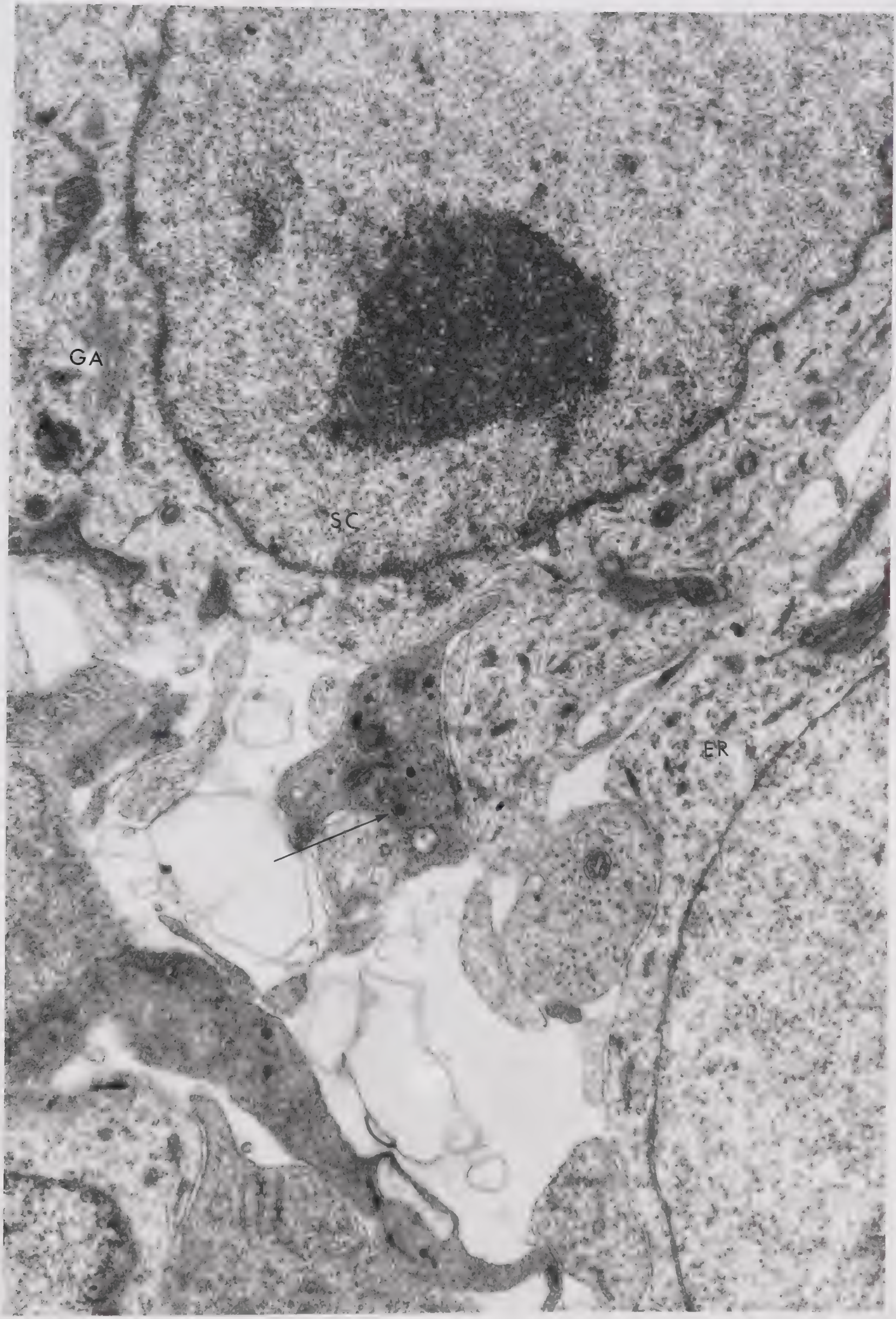

GA
SC
ER

Figure 45. A nerve ending (NE) and the processes of several photoreceptor cells (PP) in the pericapillary area. Only a few secretory granules can be seen in the process of the photoreceptor cell (↑). The nerve ending contains numerous synaptic vesicles and membrane-limited vesicles (▲), some with half-depleted dense-cores. Adult quail maintained in continuous darkness for 1 week.
RB: red blood cell; EC: endothelial cell
x 30,400

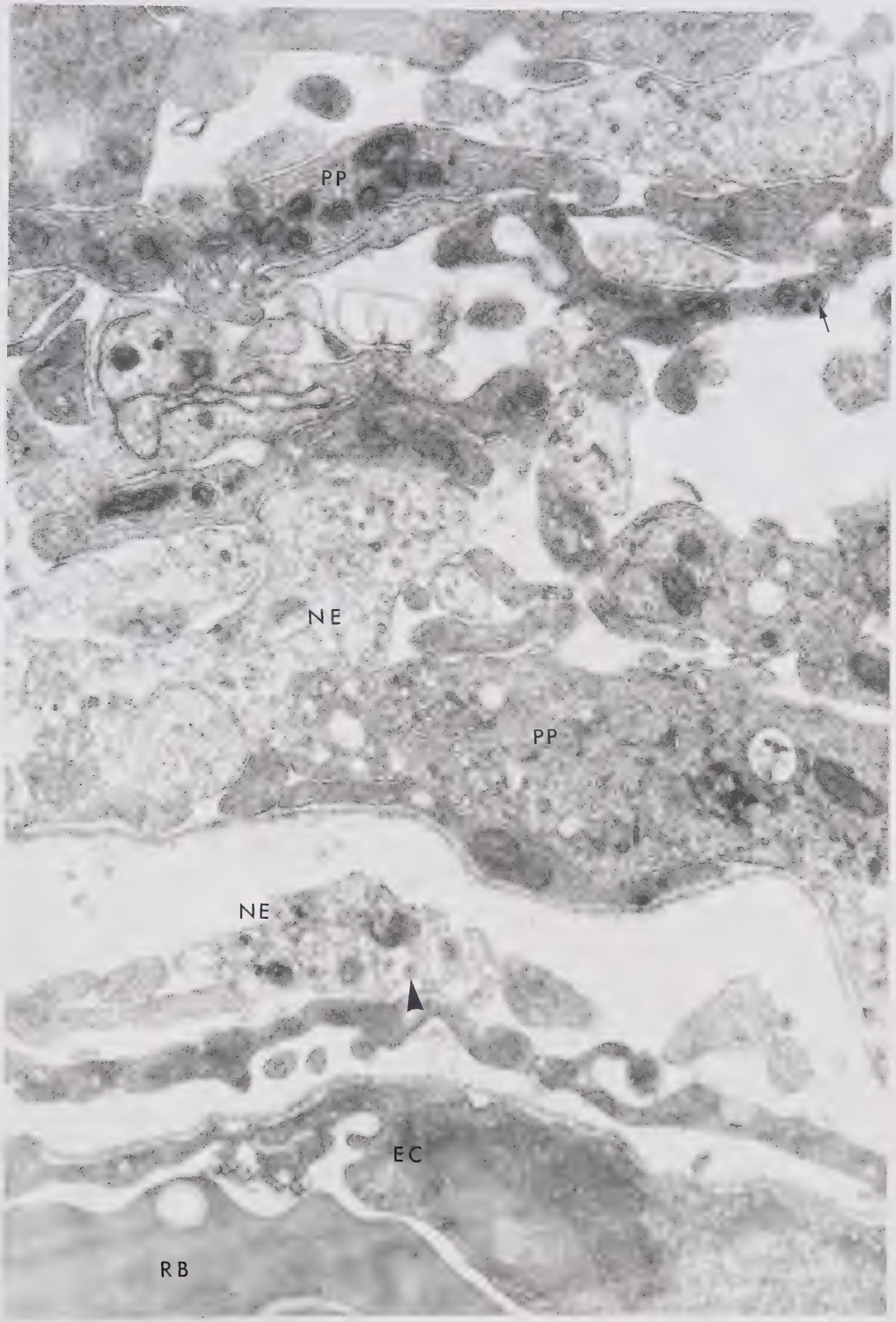

PP
NE
PP
NE
EC
RB

Figure 46. Membrane-limited dense-cored vesicles (↑) in the process of a photoreceptor cell in the lobular lumen. Adult quail maintained in continuous darkness for 5 weeks.

x 30,400

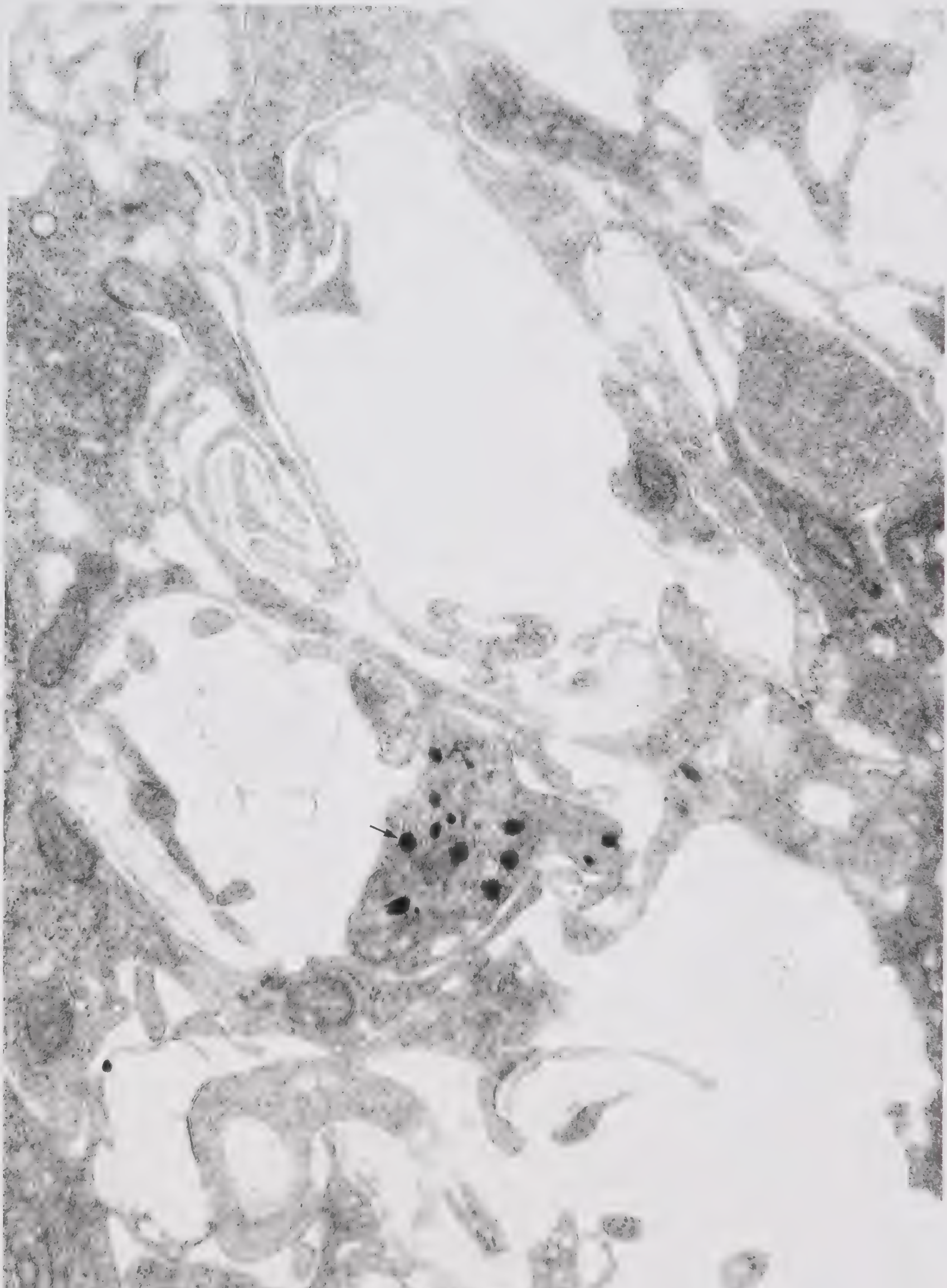


Figure 47. Processes of photoreceptor cells (PP) close to the basement membrane. The processes are devoid of secretory granules. A synaptic ribbon-like structure can be seen (A). Adult quail maintained in continuous darkness for 5 weeks.

BM: basement membrane

x 52,000

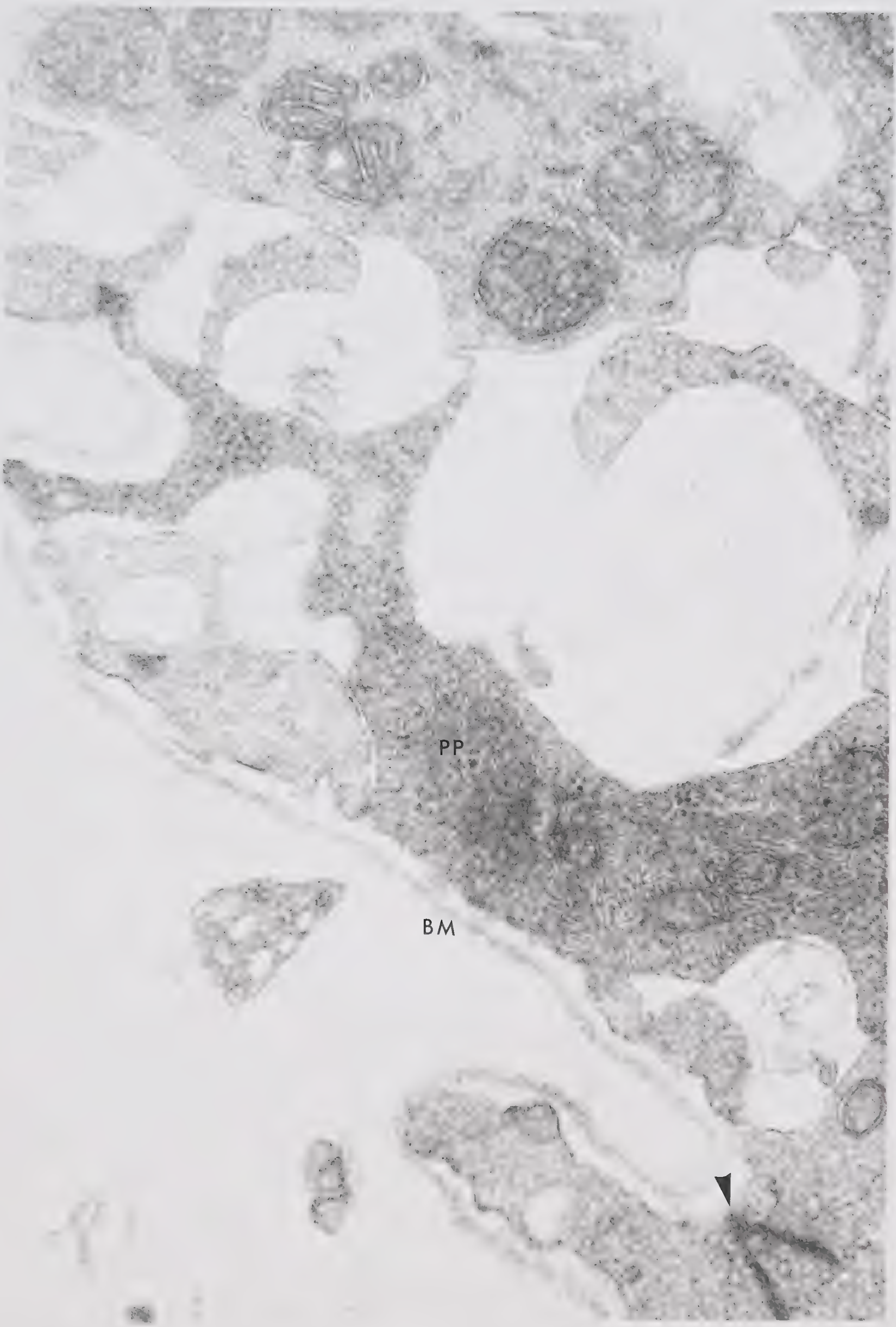

PP
BM

Figure 48. Unmyelinated nerve bundles (NB) in the pericapillary area. The photoreceptor cell processes (PP) are devoid of secretory granules. Adult quail maintained in continuous darkness for 5 weeks.

RB: red blood cell, EC: endothelial cell

x 30,400

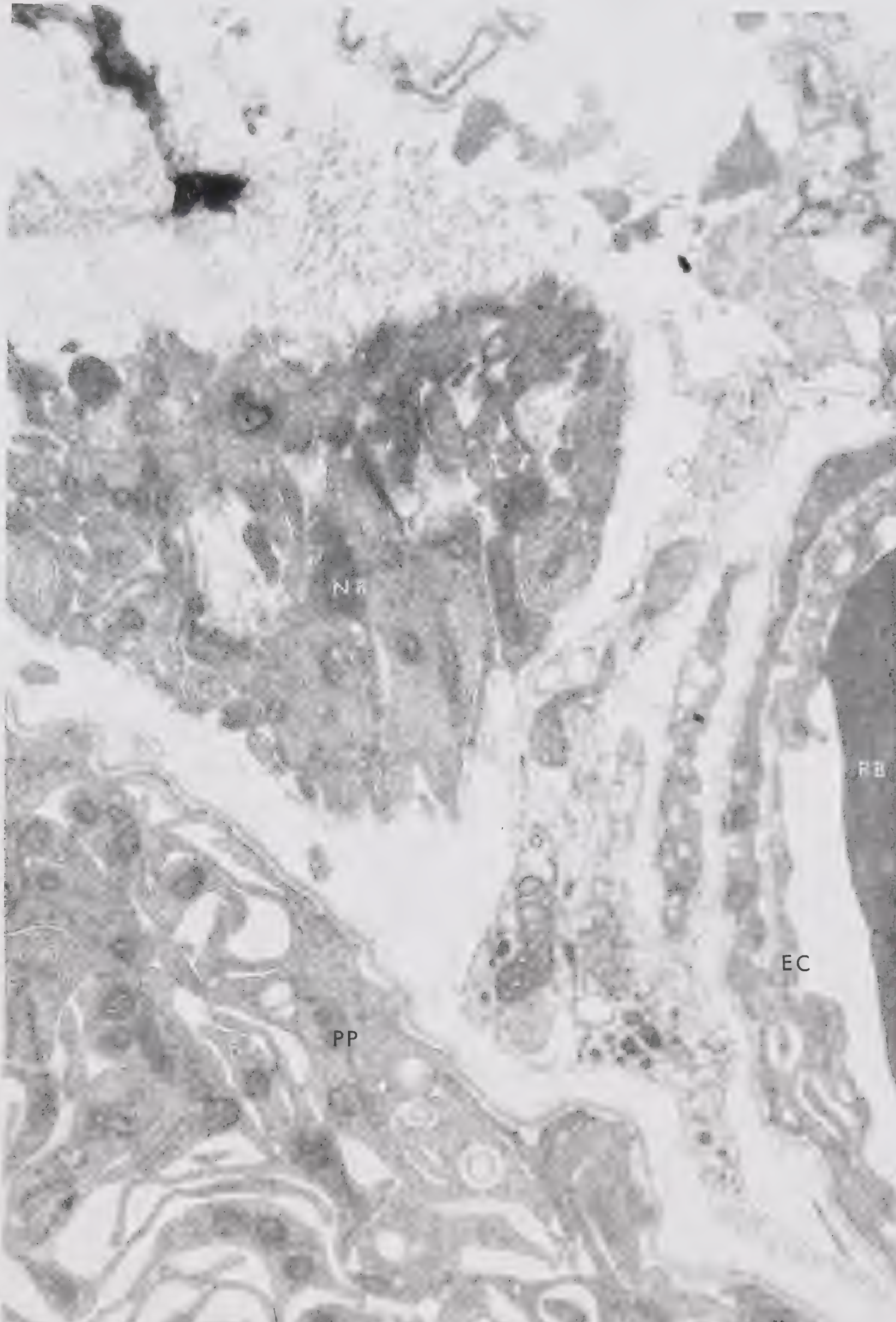
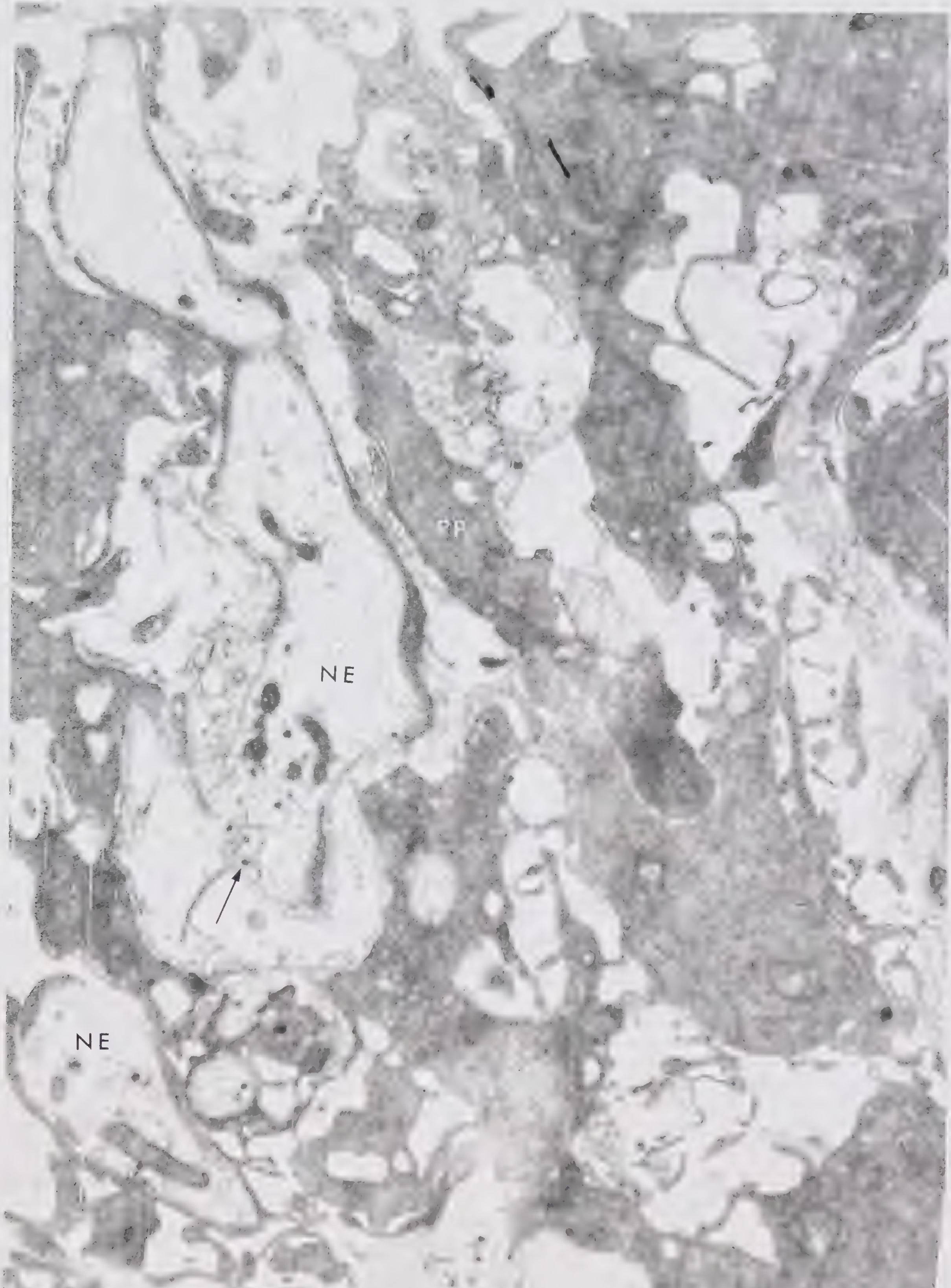

RB
EC
PP

Figure 49. Processes of photoreceptor cells (PP) and nerve endings (NE). There are no secretory granules in photoreceptor cell processes. The nerve endings contain numerous synaptic vesicles and membrane-limited vesicles with half-depleted dense-cores (↑). Adult quail maintained in continuous darkness for 5 weeks. x 18,400



PP
NE
NE

Figure 50. A nerve ending at high magnification. Note the membrane-limited vesicles with half (↑) and completely (▲) depleted dense-cores. x 52,000